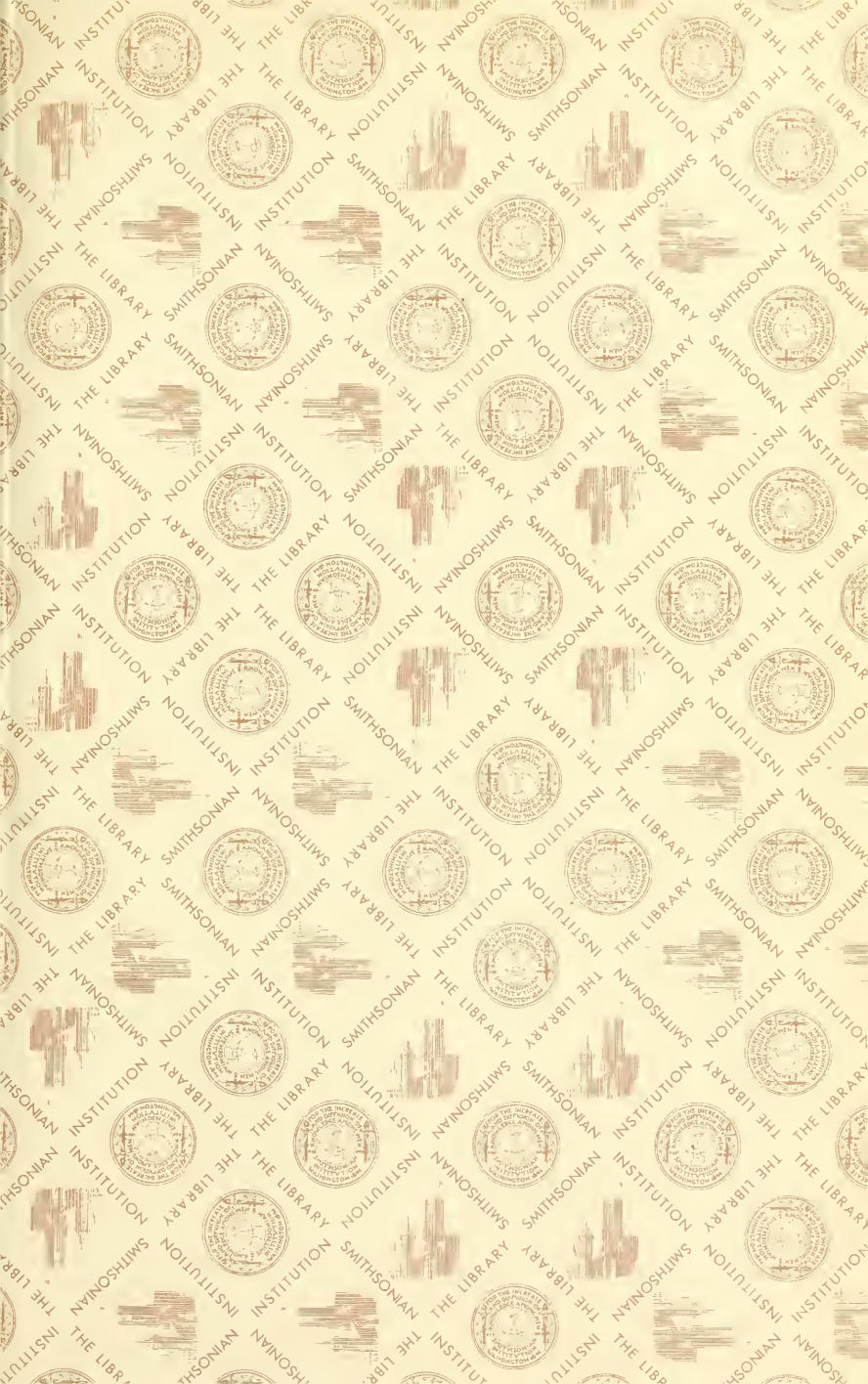
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VOLUME XX
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WITH FORTY-TWO PLATES

EDITORS
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CONTENTS

PAGES

1. Studies on the Parasites of the Termites I. On *Streblomastix strix*, a Polymastigote Flagellate with a Linear Plasmodial Phase, by Charles Atwood Kofoid and Olive Swezy; with plates 1–2; 1 figure in text 1–20

2. Studies on the Parasites of the Termites II. On *Trichomitus termitidis*, a Polymastigote Flagellate with a Highly Developed Neuromotor System, by Charles Atwood Kofoid and Olive Swezy; with plates 3–4; 2 figures in text 21–40

3. Studies on the Parasites of the Termites III. On *Trichonympha campanula* sp. nov., by Charles Atwood Kofoid and Olive Swezy; with plates 5–12; 4 figures in text 41–98

4. Studies on the Parasites of the Termites IV. On *Leidyopsis sphaerica* Gen. Nov., sp. nov., by Charles Atwood Kofoid and Olive Swezy; with plates 13–14; 1 figure in text 99–116

5. On the Morphology and Mitosis of *Chilomastix mesnili* (Wenyon). A Common Flagellate of the Human Intestine, by Charles A. Kofoid and Olive Swezy; with plates 15–17; 2 figures in text..... 117–144

6. A Critical Review of the Nomenclatnre of Human Intestinal Flagellates, *Cercomonas, Chilomastix, Trichomonas, Tetratrichomonas,* and *Giardia,* by Charles Atwood Kofoid; 9 figures in text 145–168

7. On the Free, Encysted, and Budding Stages of *Councilmania lafleuri,* a Parasitic Amoeba of the Human Intestine, by Charles Atwood Kofoid and Olive Swezy; with plates 18–22; 3 figures in text 169–198

8. Mitosis and Fission in the Active and Encysted Phases of *Giardia enterica* (Grassi) of Man, with a Discussion of the Method of Origin of Bilateral Symmetry in the Polymastigote Flagellates, by Charles Atwood Kofoid and Olive Swezy; with plates 23–26; 11 figures in text 199–234

9. The Micro-Injection of *Paramaecium*, by Chas. Wm. Rees 235–242

10. On *Balantidium coli* (Malmsten) and *Balantidium suis* (sp. nov.), with an Account of their Neuromotor Apparatus, by J. Daley McDonald; with plates 27–28; 15 figures in text 243–300

11. Mitosis in *Endamoeba dysenteriae* in the Bone Marrow in Arthritis Deformans, by Charles Atwood Kofoid and Olive Swezy; 7 figures in text 301–307

12. *Endamoeba dysenteriae* in the Lymph Glands of Man in Hodgkin's Disease, by Charles A. Kofoid, Luther M. Boyers, M.D., and Olive Swezy; 4 figures in text 309–312

13. Mitosis in the Encysted Stages of *Endamoeba coli* (Loesch), by Olive Swezy; with plates 29–31 313–332

14. The Neuromotor Apparatus of *Paramaecium*, by Charles William Rees; with plates 32–36; 5 figures in text 333–364

15. A Comparison of the Cysts of *Endamoeba coli* and *Councilmania lafleuri* in Congo Red, by Nathaniel Bercovitz, M.D. 365–371

16. On the Morphology and Behavior of *Pentatrichomonas ardin delteili* (Derrieu and Raynaud), by Charles Atwood Kofoid and Olive Swezy; with plate 37; 1 figure in text 373–390

17. The Pseudopodial Method of Feeding by Trichonymphid Flagellates Parasitic in Wood-eating Termites, by Olive Swezy; 14 figures in text 391–400

18. Methods of Obtaining Amoeba-free Rats for Experimental Infection with Intestinal Amoeba, by John F. Kessel 401–408

19. Experimental Infection of Rats and Mice with the Common Intestinal Amoeba of Man, by John F. Kessel; with plates 38–39 409–430

20. On the Genus *Councilmania*, Budding Intestinal Amoebae Parasitic in Man and Rodents, by Charles A. Kofoid, Olive Swezy and John F. Kessell; 19 figures in text 431–445

21. Morphology and Binary Fission of *Menoidium incurvum* (Fres.) Klebs, by Richard P. Hall; with plates 40–41; 2 figures in text.... 447–476

22. A Skin Reaction to Extracts of *Leishmania tropica* and *Leishmania infantum*, by Edna Hannibal Wagener; with plate 42 477–488

23. The Distinguishing Characteristics of the Parasitic Amoebae of Culture Rats and Mice, by John F. Kessel; with plates 43–48; 3 figures in text 489–544

Index 545

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Vol. 20, No. 1, pp. 1-20, plates 1-2, 1 figure in text July 14, 1919

STUDIES ON THE PARASITES OF THE TERMITES I. ON *STREBLOMASTIX STRIX*, A POLYMASTIGOTE FLAGELLATE WITH A LINEAR PLASMODIAL PHASE.

BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

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STUDIES ON THE PARASITES OF THE TERMITES I. ON *STREBLOMASTIX STRIX*, A POLYMASTIGOTE FLAGELLATE WITH A LINEAR PLASMODIAL PHASE

BY
CHARLES ATWOOD KOFOID AND OLIVE SWEZY

Smith

CONTENTS

	PAGE
Introduction	1
Material	2
Technique	3
Occurrence	4
Morphology	4
Size and shape of body	5
Cytoplasm	7
Neuromotor apparatus	7
Direction of torsion	9
Nucleus	10
Binary fission	10
Multiple fission	12
Adaptations	13
Relationships	14
Summary	16
Literature cited	17
Explanation of plates	18

INTRODUCTION

One of the most curious and unique faunal associations to be found among the parasitic Protozoa is the group parasitic or commensal in the intestinal tract of the social termites. These parasites are remarkable not only for the vast numbers that may be found within a single host, but also for the degree of development and specialization which distinguishes many of the species. This is especially true of the

forms belonging to the family Trichonymphidae, such as *Trichonympha* Leidy, which are among the most highly specialized members of the Protozoa.

Along with these more complex forms are others which, while simpler in structure, yet show certain peculiar morphological characteristics that distinguish them as a group apart from other intestinal flagellates. Among these we find *Pyrsonympha vertens* and *Dinenympha gracilis* described by Leidy in 1881.

Later investigators have added both to the number of genera and of species of these peculiar flagellates.

Material

The material for these studies was obtained from one species of termite which is abundant on the University campus at Berkeley. This is *Termopsis angusticollis* Walker and was identified for us by Dr. Nathan Banks of the Museum of Comparative Zoology at Cambridge, Mass. Most of this material was obtained from the decayed trunk of an oak tree in Strawberry Cañon. Many of the same species were obtained during the swarming season from the piles on Meiggs Wharf, San Francisco, by Dr. A. D. Drew of the Public Health Service.

These termites are large and show an infection of about one hundred per cent, soldiers, workers and males of the colony being infected alike. The amount of infection in a single individual is relatively enormous. The abdomen is large and nearly filled by the greatly swollen intestine. This distension is caused by the vast numbers of parasitic and commensal protozoans which fill the lumen of the intestine. When this is opened a thick milky fluid exudes. Under the lens this is found to be composed of great quantities of these small forms, thickly massed together, along with fragments of wood upon which the host, as well as some of its commensals, feeds.

In *Termopsis angusticollis* four different species of large protozoans are invariably present, sometimes about equal in number or with one predominating over the others. In addition to these there are usually present minute forms of two, sometimes three different species of flagellates, the whole forming a complex of organisms wonderful both for variety and amount. Of these forms the two largest species belonging to the family Trichonymphidae and a third species belonging to the Polymastigidae, will be reserved for discussion in later

papers. In the present paper the fourth member of this group, also a polymastigote flagellate, will be considered, with a discussion of its morphology, relationships and life history in so far as they have been determined.

Technique

The flagellates found in the termites are exceedingly delicate. Great difficulty has been experienced in keeping them alive for continuous observation under the microscope for any length of time. The use of distilled or tap water resulted in the complete dissolution of the larger flagellates in a few minutes. The smaller ones would survive a somewhat longer period. Various other culture media were tried, such as Ringers' solution, normal salt solution, malted milk and the white of egg. Of these the last one was the most successful, a few flagellates surviving in the culture at the end of twenty-four hours. These cultures were made with a hanging drop or with a greater amount of fluid in a deep culture slide.

Intra vitam stains, such as neutral red, Janus green and new methylene blue G G, were used. Of these neutral red gave the best results.

The methods of fixing and staining which have been found the most satisfactory were those outlined by us in previous work on parasitic flagellates (Kofoid and Swezy, 1915), that is, a modified Heidenhain's iron haematoxylin following fixation in hot Schaudinn's fluid. Other stains as well as various fixing agents were tried, both with smear preparations and with sectioned material. In the latter cases two methods were followed. In the first the entire abdomen of the termite was used, fixed in Schaudinn's or strong Flemming's solution. In the second the intestine was teased out in a drop of normal salt solution and then placed in the fixing fluid. These sections were stained with haematoxylin or with a modified Mallory's connective tissue stain (Yocom, 1918).

Considerable difficulty has been experienced in making good preparations of this material by the ordinary smear methods. The exceeding delicacy of the various flagellates results in distortions of the body and breaking down of its cytoplasmic organization. This is usually confined to the posterior end, leaving the anterior end, nucleus and motor organelles intact. This difficulty was partly overcome by using albumen fixative on the cover slip and diluting the contents of the

intestine with a small drop of normal salt solution before making the smear. The material thus treated may be spread with less mechanical injury and the albumen prevents the great loss of organisms that would otherwise occur when it is placed in the fixing fluid. The addition of albumen, however, necessitates quick work in making the preparations, to prevent the death of the organisms through its action rather than that of the fixing fluids.

OCCURRENCE

These flagellates are more restricted in their occurrence in the intestine of the host than are the other forms which are present with it. They are seldom found far away from the mucus of the epithelium, usually attaching themselves to it (pl. 1, fig. 9) by means of the holdfast-like anterior end of the body. They may be seen completely filling the folds of the wall of the intestine with the posterior portion projecting into the lumen of the canal.

Near the anterior part of the posterior region of the intestine, immediately behind the origin of the malpighian tubules, a slight enlargement of the intestine may be noted, with one side marked by two lines of constriction passing backward for a short distance. This forms a rounded chamber marked off from the main portion of the canal. In cross-sections this may be found completely filled with *Streblomastix*, a dense coating of the flagellates attached to the wall and others filling the remainder of the cavity. Plate 2, figure 8 shows a small portion of the wall in this region with a few only of the attached flagellates.

These flagellates occur much less frequently in other parts of the posterior and mid-regions of the intestine, but when present are always restricted to the peripheral zone with the larger flagellates occupying the remainder of the lumen. They have been found in nearly seventy per cent of the hosts examined.

MORPHOLOGY

Streblomastix is profoundly a linear organism. Elongation dominates all of its organelles in adaptation to its crowded grouping in its parasitic habitat. This elongation affects not only the body as a whole but also the nucleus, rhizoplast, and flagella, and pervades not only the normal vegetative trophozoite, but also the gigantic over-

grown and possibly abnormal phases occasionally found. The linear form of body and also of nucleus continues not only during the trophozoite phase but likewise, in so far as we have seen the stages, during both binary and multiple fission. During multiple fission itself the organism becomes a greatly elongated thread with its nuclei stretched lengthwise as a constricting thread in the axis of the body. All trace of rounding up or sphericity seems thus to have been banished utterly from both body and nucleus at all stages of its life cycle.

Size and Shape of Body

The body is ordinarily elongate fusiform, tapering subequally at the two ends. Either or both ends (pl. 1, figs. 1, 7) may be somewhat blunt but the usual form of the anterior end is a slender cone while the posterior one may have a trifle more convexity. Its length is generally from twelve to sixteen times its greatest diameter which is found near the middle of the body. The shorter individuals (pl. 1, fig. 5) may be only six times the diameter. These are evidently recent schizonts. On the other hand, giant individuals, which are possibly approaching multiple fission (pl. 2, fig. 13), may be thirty times their diameter in length, and the "plasmodial" stage of multiple fission (pl. 2, fig. 14) attains a length as much as seventy times its own diameter. Measurements of two hundred individuals gave a frequency curve with a marked left-hand skew with the mode at 40μ and the extreme range in length of from 20 to 530μ. One-half of the individuals were included between 20 and 80μ. The longest individuals included those in which multiple fission was in progress and it is probable that the others were approaching that phase.

The contour of the body is not a smooth line, for the surface is traversed by spiral ridges with furrows between, giving it the form, except for its taper, of the shaft of a Norman Romanesque column. These ridges are four in number, broadly convex and equidistant and they wind about the body from the anterior end posteriorly from the left over to the right. It is thus like a left-hand screw if the anterior end is regarded as the tip. The steepness of the spiral varies with the length of the organism, its contraction, and the phase of the life cycle. In late stages of binary (pl. 2, fig. 17) and multiple fission (pl. 2, fig. 14) much of the torsion is relaxed. In stages which may be prior to binary fission (pl. 1, fig. 7; pl. 2, fig. 10) these may be

three to five turns, and a giant individual, presumably preceding multiple fission (pl. 2, fig. 13), has eight turns. Normal vegetative individuals (pl. 1, figs. 1, 6, 8) have one to two turns only.

The grooves between the ridges mark the location of ectoplasmic lines of deeply staining material, possibly myonemes, or extensions

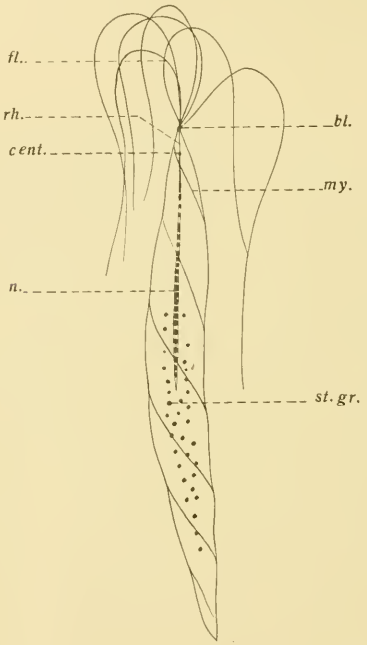

Fig. A. Semidiagrammatic figure of *Streblomastix strix* gen. nov., sp. nov. Black granules in cytoplasm show particles stained *intra vitam* with neutral red. Abbreviations: *bl.*, blepharoplast; *cent.*, centrosome; *fl.*, flagella; *my.*, myonemes; *n.*, nucleus; *rh.*, rhizoplast; *st. gr.*, stained granules. × 2000.

of the neuromotor system. They are feebly stained, if at all, in binary and multiple fission (pl. 2, figs. 14, 17, 19) and are sometimes marked by accumulations of minute granules staining black with haematoxylin. They terminate anteriorly at or near the blepharoplast and fade out posteriorly.

Cytoplasm

The cell contents are undifferentiated. No separation of ectoplasm, pellicle, and endoplasm is visible. There is no cytostome and no food particles or food vacuoles have been detected. There is no contractile vacuole visible. The only differentiated structures normally visible in the organism are the nucleus and the neuromotor apparatus. However, on treatment with neutral red (fig. A) certain granules became stained in the axial cytoplasm in the posterior two-thirds of the body, indicating an endoplasmic territory within which digestion of the absorbed food was in progress.

Neuromotor Apparatus

This neuromotor apparatus consists of a centrosome, rhizoplast, blepharoplast, myonemes, and six flagella. The centrosome (fig. A, *cent.*; pl. 1, fig. 9; pl. 2, fig. 10) is found at the anterior tip of the elongated nucleus. It appears to be a spherical granule in or on the nuclear membrane. It is temporary or evanescent and does not appear to play any visible part in either binary or multiple fission. It is quite possible that the granule thus interpreted is a mere temporary accumulation of chromatoidal substance on the rhizoplast without morphological meaning. Its position with reference to nucleus, rhizoplast and blepharoplast is similar to that of the centrosomes in *Giardia* (Kofoid and Christiansen, 1915, and Boeck, 1917) hence the designation suggested. It may be that the extrusion of the rhizoplast serves to bring the structure into view from a more intimate union with the nuclear membrane.

The rhizoplast (fig. A, *rh.*) is a slender, deeply staining thread running anteriorly from the centrosome or the anterior tip of the nucleus to the blepharoplast. Its length ordinarily is about equal to the greatest diameter of the body and the line of demarcation between the attenuate end of the nucleus and this thread is often not readily determined. There is a probability that this is more or less contractile, as is seen on a comparison of our figures. In one instance (pl. 1, fig. 7) this structure appears to be foreshortened and thickened, by contraction, onto the anterior end of the nucleus.

One remarkable feature of this structure is its capacity of being extended beyond the anterior end of the body as a long spike bearing the blepharoplast and its attached flagella at its tip. A con-

siderable increase in length to nearly three or even six times the normal seems possible (pl. 1, fig. 3). No evidence of an extension of the protoplasmic pellicle to form a sheath for this remarkable organ has been found. It may also be so foreshortened that the blepharoplast and centrosome are brought into close juxtaposition (pl. 1, fig. 2).

In view of the fact that the individuals lie closely packed against the digestive epithelium with the blepharoplast thrust against the epithelial cell of the host, it appears that this protrusible organ serves in some way as a part of a somewhat adjustable holdfast. We have no evidence that it can be or is ever thrust into the body of the cell, though such a mode of attachment seems possible.

The blepharoplast (fig. A, *bl.*)is a sphere at the anterior end of the rhizoplast about 0.5μ in diameter. The six flagella spring directly from it. It lies normally in the extreme anterior end of the body and is carried out with the extruded rhizoplast. The possibility of its being drawn out in detaching the parasite from its adhesion to the cells of the host is not precluded, but the numbers of such cases and the retention of normal symmetry of both body and rhizoplast does not support the suggestion of forceable extraction:

In one instance (pl. 1, fig. 4) a terminal blob of cytoplasm with a deeply staining terminal cap is attached to the side of the blepharoplast, and in another a considerable mass of protoplasm lies about the extruded blepharoplast. While these may be abnormalities it is possible that under certain conditions the cytoplasm assists locally in the holdfast function of the blepharoplast by forming an enlarged mass about it.

The six flagella are equal, habitually trailed posteriorly and about half as long as the body. They serve to keep up the circulation of the fluid contents of the digestive tract as they lie parallel to the closely packed bodies of the parasites (pl. 1, fig. 8) in the folds of the digestive epithelium.

The four peripheral spiral threads (fig. A, *my.*) which terminate at or near the blepharoplast must be regarded as a part of the neuromotor apparatus. Their relations to the blepharoplast and their stainability as well as the homology suggest this. Their function, if contractile (and their spiral course indicates this), appears to be to force the blepharoplast into intimate contact with the cells of the host. They persist at cytolysis and individuals are often found (pl. 1, fig. 10) in which these myonemes are frayed out as distinct lines.

These myonemes are evidently quite firm fibers, somewhat elastic and more or less rigid. They often stain very deeply especially in disintegrating individuals (pl. 1, fig. 1).

Direction of Torsion

The direction of torsion of these elements of the neuromotor apparatus is not without a deep significance. It is the same as that of the undulating membrane or attached flagellum of *Trichomonas*, *Trichomitus*, *Tetratrichomonas* and *Eutrichomastix* (Kofoid and Swezy, 1915), other polymastigotes in which torsion finds some structural expression. This same direction of torsion appears in the myonemes of *Pyrsonympha* and *Dinenympha* (Leidy, 1881). Grassi and Sandias (1893) reverse the direction of *Pyrsonympha* and of *Holomastigotes* in their figures, while Porter (1897) figures both directions. It is perhaps significant that his figures from life have the reversed direction while those from preparations, and therefore presumably accurate, have the normal leiotropic, or to be expected, direction. The reversals figured by Grassi and Sandias require confirmation before acceptance. The *Pyrsonympha* of this contribution (1893) is later designated as *Spirotrichonympha* by Grassi and Foà (1911) but without note of the differences in torsion. Zulueta (1915) figures the leiotropic direction in what appears to be Grassi's *Spirotrichonympha*. While it is quite possible that both leiotropic and dexiotropic genera or species exist, or that functional reversals of torsion occur in the individual it is even more evident that critical observations are essential to establish these diametrically opposed conditions. Pending such investigations the preponderance of the evidence favors the view that the torsion of the more primitive Trichonymphidae is leiotropic, that is from right over to left posteriorly, as it is in *Streblomastix*.

This is also the fundamental direction of the girdle and of the encircling transverse flagellum of the Dinoflagellata, and also of the attached collar-forming, ribbon-like flagellum of the Craspedomonadina (Burck, 1909). These facts are suggestive of an extensive and deep-seated leiotropism in the organization of the Mastigophora which finds expression in both externally attached flagella and internal contractile myonemes. That it may be conditioned by some equally pervasive stereometric properties of certain compounds of the living substance seems plausible.

Nucleus

The nucleus (fig. A, *n.*) shares the elongation which affects the body and appears to be pulled far anterior by the holdfast function of the blepharoplast so that in comparison with other polymastigotes its location is exceptionally far anterior. Its length is from 0.3 to 0.5 that of the body itself and its shape is fusiform but much more slender than the body, its length being fifteen to twenty-five times its diameter. It tapers about equally at both ends and appears in most of our preparations as a solid black axial strand in the anterior part of the body. Unless very strongly decolorized no internal structures can be made out. It appears to be composed of almost solid chromatin. When sufficiently decolorized (pl. 1, figs. 7, 9) a distinct nuclear membrane is evident within which a single row of black chromatin spherules, decreasing in size towards each end, can be detected. These are not uniform in size or arrangement and are about twenty-five in number. They are not unlike the chromomeres which we have found in the chromosomes of *Trichonympha*.

BINARY FISSION

The life history of *Streblomastix* presents those phases of development which we (Kofoid and Swezy, 1915 and Kofoid and Christiansen, 1915) have previously described for other polymastigotes, namely, binary and multiple fission. As yet no encystment has been detected and no indications of sexual reproduction. The differences in size which we find would doubtless some years ago have afforded a basis for the speculative designation of microgametes and macrogametes and the corresponding gametocytes as well as for the predication of sex, as Hartmann (1910) did in the case of *Trichonympha*. However, in the absence of evidence of *sexual behavior* and observed fusion of gametic nuclei, the free swing of such speculation is wisely held in abeyance.

Binary fission occurs in the trophozoite stage. There is some evidence that it is cyclic since many individuals in approximately the same stage of mitosis will be found in a single host. It is not, however, restricted wholly to such cycles since isolated cases of fission have been found and not all individuals parasitic in one host are in fission at one time. Successive infections and diverse stocks of the parasites doubtless exist in the host and may afford the occasion for this diversity.

Unfortunately our material, though extensive, has not given us all of the stages of nuclear behavior during fission so that we are unable to trace wholly, the successive phases of mitosis. We have found no clear evidence of chromosome formation, beyond the twenty-five or more spherical aggregates of chromatin in the linear nucleus. We have found no spherical stage of the nucleus, no skein, and have not detected the division of the blepharoplast which doubtless occurs, neither have we been able to find the paradesmose spun out between the daughter blepharoplasts (pl. 2, figs. 12, 13).

The process of binary fission, in so far as our partial evidence goes, takes place without any rounding-up of the elongated body. The anteriorly located blepharoplast divides, new flagella arise from one or both of the daughters, and one migrates to the opposite end of the body (pl. 2, fig. 11). In the meantime the nucleus has become greatly elongated, reaching from end to end of the body. It then constricts at the middle (pl. 2, fig. 12), finally parts there (pl. 2, fig. 19) and the two schizonts separate. To all appearances this is *transverse division*. Longitudinal division is, however, the fundamental and universal method of binary fission in the Euflagellata as compared with the Ciliata in which transverse division occurs. This seeming departure from the normal is, however, more apparent than real, for if the anterior blepharoplast divides and one daughter migrates to the posterior end we will have such an arrangement of schizonts as in *Trichomonas* after mitosis but before plasmotomy (Kofoid and Swezy, 1915, pl. 4, fig. 39). This is a temporary relation in such a metabolic form as *Trichomonas* but a more lasting one in *Strcblomastix*. The mode of division is therefore still morphologically longitudinal though almost the last vestige of the appearance of that type of division has been submerged by the dominating elongation of the body in *Strcblomastix*. While it is possible that there is a series of skein-chromosome changes in the nucleus which has escaped us, our present evidence indicates that these are also suppressed or hidden in the dense chromatin threads which part by simple median constriction (pl. 2, fig. 12). This parting is delayed until the posterior daughter blepharoplast is in its final position, as in other polymastigotes (Kofoid and Swezy, 1915). The frequent occurrence of stages in schizogony with the nucleus as yet undivided or dividing, but the neuromotor organelles in duplicate, indicates that both nuclear constriction and plasmotomy following thereon are prolonged processes.

MULTIPLE FISSION

Multiple fission in *Strcblomastix* is a cyclic process occurring in many individuals in a single host at one time. A few only may be found in this stage, or, in some instances, at least, the majority of individuals may be in the multinucleate phase.

This condition is preceded by the growth of the schizont from the small size resulting from multiple (pl. 2, fig. 13) or binary (fig. 17) fission to a much larger or even giant stage (pl. 2, fig. 13), which may be as much as twenty-six times the length of the smallest schizont. The body may have as much as sixty times the mass of the smallest stages. The nucleus, however, does not increase proportionately, remaining, in fact, at least in many instances, almost unchanged (pl. 2, fig. 13).

At some period during this increase in size multiple fission sets in. Not all trophozoites entering upon it attain the maximum size as will be seen on a comparison of figures 15 and 16 on plate 2. It is possible that figure 16 represents only a detached section of a larger plasmodium which is fragmenting, or it may be a small trophozoite in the initial stages of multiple fission.

Contrary to the behavior of the blepharoplast-flagella complex in binary fission where it leads in division, preceding the nucleus, we find that in multiple fission nuclear division by transverse constriction is taking place prior to the division of the blepharoplast (pl. 2, figs. 14, 15). The type of nuclear division is the same as in binary fission. We have not seen stages of multiplication of the blepharoplast or of plasmotomy.

The linear form persists during the period of multiple fission and the nucleus becomes an elongated axial chromatin thread which becomes attenuate locally and parts transversely (pl. 2, figs. 13–16). The number of nuclear segments varies, probably in a 2–4–8 sequence, although irregularities in this are apparent. The largest number observed is eight. This accords with multiple fission in other polymastigotes (Kofoid and Swezy, 1915; Kofoid and Christiansen, 1915).

There is some evidence that this stage is contractile and that when foreshortened the nuclei slip by one another. This is not a common condition and probably does not represent a rounded-up condition obligatory for multiple fission but rather a passing response to stimulus resulting in contraction.

Multiple fission stages which we have seen provide for eight schizonts when the plasmodium parts by plasmotomy into its constituent zooids. From irregularities in the number of nuclei in preparations in which multiple fission is common, it seems probable that plasmotomy is an irregular dropping off of individuals or groups of individuals from the common mass as in *Trichomonas*. We have no evidence as to the presence of a centrosome during multiple fission and none as to the origin of the new blepharoplast-flagella complexes of the daughter schizonts.

ADAPTATIONS

Although seemingly simple in structure *Strcblomastix* presents a series of structural adaptations which in the light of its parasitic mode of life become significant of intimate correlations with the conditions under which it exists and its habits. The entire loss of the cytostome is associated with feeding by osmosis and results in the disappearance of the bilateral asymmetry characteristic of polymastigotes such as *Trichomonas*. The absence of large food particles makes possible the elimination or reduction of cytosis of the endoplasm and facilitates the change of form to a long and relatively very narrow spindle within which such movement would be impeded. The spiral course of the myonemes or spiral striae provides a most effective form of mechanism for an energetic thrust of the holdfast blepharoplast against or into the cells of the host. It is also a form of contraction which would disturb but slightly the closely packed grouping of the parasites. The spirally fluted surface combined with the action of the posteriorly directed flagella would give rise to vortex currents of the circumambient digestive fluids and thus provide the circulation essential to the metabolism of the parasite while at the same time permitting their segregation apart from the other organisms of the digestive tract of the host. The contractile extrusible rhizoplast-blepharoplast complex with its blob of cytoplasm affords an efficient structural holdfast. The elongation of the nucleus provides a spatially advantageous grouping for the nucleo-cytoplasmic interchanges in the absence of marked cytosis.

The neuromotor apparatus is so arranged as to give well distributed contact with the surrounding medium by means of flagella and striae and with the host by means of the blepharoplast-rhizoplast, while this in turn is connected with the nucleus, thus establishing the structural essentials for efficient coördination of functions.

Even the reproductive phases of binary and multiple fission retain the elongated form characteristic of the trophozoite, thus permitting those stages to retain their position among the segregated parasites of their own kind. In other polymastigotes, such as *Trichomonas*, the stage of multiple fission is an amoeboid plasmodium, a rounded-up, somewhat shapeless amoeboid mass (Kofoid and Swezy, 1915). In *Streblomastix*, however, the linear form persists throughout this phase, in so far as we have observed it, although it shows greater laxity of form and less torsion (pl. 2, figs. 14, 15) than do the vegetative trophozoites. Thus in every feature of its structure and phase of its life history *Streblomastix* is intimately adapted to its peculiar parasitic mode of life notwithstanding its seeming simplicity of structure.

RELATIONSHIPS

The presence of six flagella definitely allocates *Streblomastix* in the Order Polymastigina. Its relative simplicity of structure as compared with most genera of this order is shown by the undifferentiated condition of the flagella. There is no single specialized trailer attached as an undulating membrane, and none intracytoplasmic as an axostyle.

The presence of the four longitudinal spiral ectoplasmic "myonemes" or extensions of the neuromotor apparatus is very suggestive of a relationship to the Trichonymphidae in most of which such lines arise from the blepharoplast and are the stems from which spring the many so-called cilia. This relationship will be more evident on detailed comparison. There are eight such lines in *Dinonympha gracilis* (Leidy, 1881) along the course of each of which small cilia take their origin, but there are no developed anterior flagella. Zulueta (1915) has shown that these extend posteriorly as free flagella and that they are grouped four and four on the daughter centrosomes at the poles of the spindle at mitosis. The species upon which Zulueta worked appears to be the same as that figured by Grassi and Sandias (1893, pl. 5, figs. 18–20) which Grassi and Foà (1911) later distinguish from Leidy's species as *Spirotrichonympha*. *Pyrsonympha vertens* (Leidy, 1881 and Porter, 1897) likewise has eight such lines arising from the blepharoplast with small lateral cilia arising from them. Anteriorly there is a blepharoplast from which a free slender thread extends anteriorly into the host cell not unlike the rhizoplast-blepharo-

plast of *Streblomastix* in superficial appearance, but possibly homologous with flagella and derived by modification from one or more of them. There are four such lines in *Holomastigotes* (Grassi and Sandias, 1893) giving rise to lateral cilia and pursuing a spiral course posteriorly from what is probably an anterior blepharoplast. There are, however, no large anterior flagella arising from this point.

The form most nearly allied to *Streblomastix* appears to be *Pyrsonympha* by reason of the persistence of an anterior outgrowth from the blepharoplast which may be homologized with flagella. However, it has lateral cilia arising from its spiral lines. These *Streblomastix* entirely lacks. This absence of the lateral ciliary coat justifies the exclusion of *Streblomastix* from the Trichonymphidae and renders its retention in the Polymastigina necessary. However, it may be regarded as closely related to that branch of the polymastigote stock from which the Trichonymphidae originated. *Streblomastix* thus forms a living link between the Polymastigina and the Trichonymphidae, linking the latter seemingly aberrant forms more closely and definitely than heretofore to the Flagellata as its most highly specialized order.

It is obvious that *Streblomastix* can not be allocated in the family Hexamitidae and that its relationships with the Polymastigidae are relatively remote. Even its inclusion in the Polymastigina is somewhat problematical. Its transfer to the Trichonymphidae is defensible but requires a profound modification in the definition of that order. To set forth more strongly its intermediate position we have left it in the Polymastigina and propose for it a new family, the Streblomastigidae, as follows:

Family Streblomastigidae fam. nov.

Polymastigina with spiral myonemes and anterior flagella.

Streblomastix gen. nov.

Streblomastigidae with six anterior flagella and four leiotropic myonemes. Type species *Streblomastix strix* sp. nov. from *Termopsis angusticollis* Walker.

SUMMARY

1. *Streblomastix strix* occurs as an intestinal parasite of the termite *Termopsis angusticollis* and is usually found attached to the epithelium of the intestine posterior to the malpighian tubules, segregated from the other parasites in the lumen.

2. It is a linear organism with the nucleus elongated to conform to the shape of the body. Its neuromotor apparatus consists of centrosome, blepharoplast, four myonemes and six flagella, connected with the nucleus by the rhizoplast.

3. Binary fission apparently occurs without spindle formation. The nucleus elongates and becomes constricted prior to the constriction of the protoplasmic body.

4. Multiple fission is a cyclic process occurring in many individuals in a single host at one time. It may be preceded by the formation of giant individuals. The body retains its linear formation throughout the process as in binary fission. The nucleus elongates and constricts into daughter nuclei. The greatest number observed is eight.

5. The shape of the body and the arrangement of its neuromotor apparatus show striking adaptation to the habitat in which the flagellate is found and to its parasitic mode of life.

6. *Streblomastix* forms a living link between the Polymastigina and the Trichonymphidae but without close relations in either group. We therefore propose for it a new family, Streblomastigidae, which we place in the Polymastigina.

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Berkeley, California.
Transmitted September 5, 1918.

LITERATURE CITED

Boeck, W. C.
1917. Mitosis in *Giardia microti*. Univ. Calif. Publ. Zool., **18**, 1–26, pl. 1.

Burck, C.
1909. Zur Kenntnis der Histologie einiger Hornschwämme, sowie Studien über einige Choanoflagellaten. (Heidelberg, Rossler), 61 pp., 2 pls.

Grassi, B., and Foà, A.
1911. Intorno ai Protozoi dei Termitidi. Nota preliminare. Rend. R. Accad. dei Lincei, Cl. Sci. Fis. Mat. e Nat., Rome, (5), **20**, 725–741.

Grassi, B., and Sandias, A.
1893. Costituzione e sviluppo della societa dei Termitidi. (Catania Galatola), 150 pp., 4 pls.

Hartmann, M.
1910. Untersuchungen über Bau und Entwicklung der Trichonymphiden (*Trichonympha hertwigi* n. sp.). Festschr. z. Hertwigs, **1**, 349–396, pls. 27–30, 3 figs. in text.

Kofoid, C. A., and Christiansen, E. B.
1915. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–34, pls. 5–8, 1 fig. in text.

Kofoid, C. A., and Swezy, O.
1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad. Arts. Sci., Boston, **51**, 289–378, pls. 1–8, 7 figs. in text.

Leidy, J.
1881. The parasites of the termites. Jour. Acad. Nat. Sci., Philadelphia, (2), **8**, 425–450, pls. 51, 52.

Porter, J. F.
1897. *Trichonympha* and other parasites of *Termes flavipes*. Bull. Mus. Comp. Zool., Cambridge, **31**, 48–68, pls. 1–6.

Yocom, H. B.
1918. The neuromotor apparatus of *Euplotes patella*. Univ. Calif. Publ. Zool., **18**, 337–396, pls. 14–16.

Zulueta, A. de
1918. Sobre la reproduction de *Dinenympha gracilis* Leidy. Trab. Mus. Nac. Ciene. Nat., Madrid, ser. Zool., **23**, 15 pp., 1 pl., 6 figs. in text.

EXPLANATION OF PLATES

All drawings of *Streblomastix strix* were made with camera lucida from material stained with iron haematoxylin, with a magnification of 2500 unless otherwise stated.

PLATE 1

Fig. 1. Ordinary trophozoite showing few turns in torsion of the body.

Fig. 2. Contracted specimen showing centrosome and blepharoplast near together.

Fig. 3. Giant individual not fully drawn. Note elongated rhizoplast with blob of cytoplasm surrounding blepharoplast.

Fig. 4. Individual showing definite nuclear membrane and blob of protoplasm with chromatin cap attached to the blepharoplast.

Fig. 5. Body slightly contracted with little torsion.

Fig. 6. Myonemes showing heavily stained lines.

Fig. 7. Trophozoite with considerable torsion of body.

Fig. 8. Position of parasites in villi of intestine; attached to the mucous lining but not to the cells of the wall.

Fig. 9. Trophozoite with greatly elongate rhizoplast.

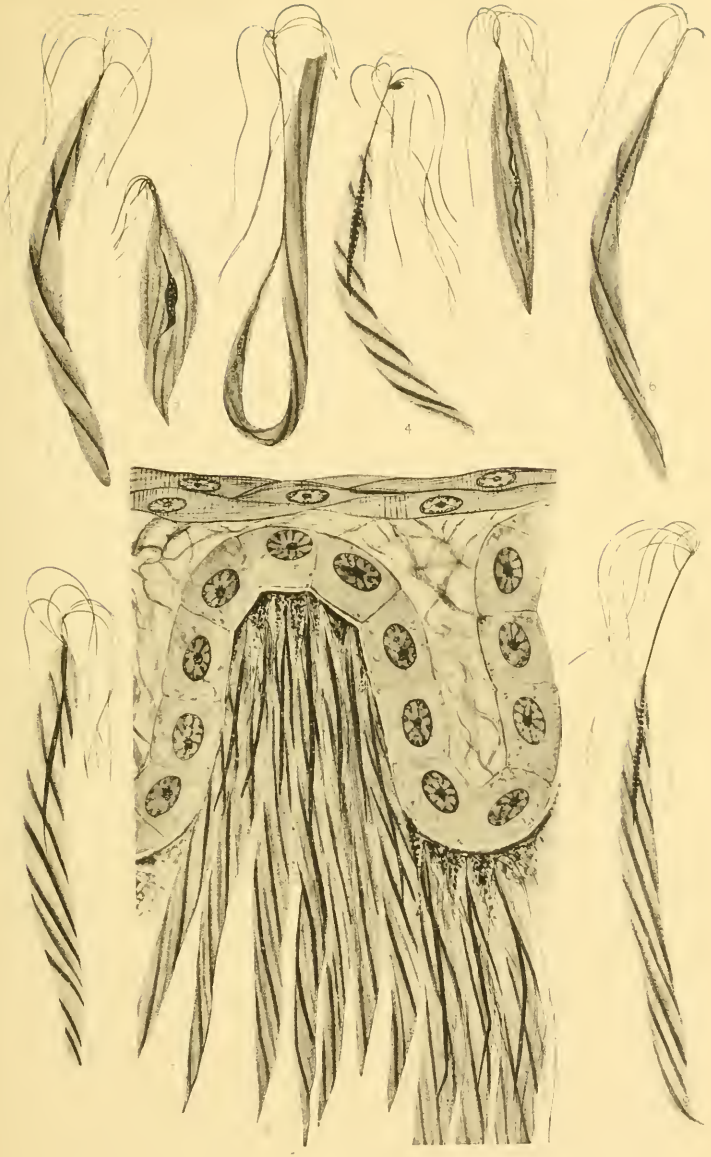
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PLATE 2

Fig. 10. Division form. Note length and structure of nucleus.

Fig. 11. Individual with myonemes fraying out from the protoplasmic groundwork.

Fig. 12. Binary fission with the nucleus constricting.

Fig. 13. Giant individual.

Fig. 14. Multiple fission with nucleus constricted in the formation of seven or eight schizonts.

Fig. 15. Multiple fission form preceding constriction of nucleus.

Fig. 16. Multiple fission form with three schizonts.

Fig. 17. Telophase of binary fission.

Fig. 18. Individual in process of fission.

Fig. 19. Final stage of binary fission; parting of schizonts.

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BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

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CONTENTS

	PAGE
Introduction	21
Occurrence	22
Morphology	23
Shape and size of body	23
Cytoplasm	24
Cytostome	26
Neuromotor system	26
Nucleus	29
Mitosis	31
Binary and multiple fission	35
Encystment	36
Relationships	36
Summary	37
Literature cited	37
Explanation of plates	38

INTRODUCTION

The occurrence in polymastigote flagellates of a structurally integrated fibrillar complex consisting of centrosome, rhizoplast, blepharoplast, axostyle, undulating membrane, parabasal body, and flagella in *Trichomonas* was described by us (1915) and the complex designated as an extra-nuclear motor apparatus. Its analogy to the neuromotor apparatus of the ciliate *Diplodinium* was noted.

In the following year the more highly specialized and intimately integrated fibrillar apparatus of the binucleate diplozooic *Giardia* was definitely designated (Kofoid and Christiansen, 1916) as the neuromotor apparatus. In a paper read before the Second Pan-American Scientific Congress at Washington, January 7, 1916, the senior author extended the neuromotor conception to the flagellates generally to include the centrosome-blepharoplast and its external and internal fibrillar derivatives and connections under the name of the neuromotor apparatus.

It is the purpose of this paper to describe the neuromotor apparatus or system of one of the simpler trichomonads in which there is no axostyle but in which there occurs in response to the parasitic habit an exceptionally massive development and structural continuity of the several elements of this coördinating organ system. The organism also presents a prophetic prolongation of the period of existence of the paradesmose and of the ineipient stage of mitosis,—features which are strongly suggestive of a tendency which, if continued, might well culminate in the evolution of the diplozooic flagellates, such as *Giardia*. The potency of the biochemical environment of parasites in bringing to expression latent possibilities of the organization of the living substance is once again demonstrated in this flagellate of those extraordinarily parasitized insects, the termites.

OCCURRENCE

This flagellate has been found abundantly in *Termopsis angusticollis* Walk., a large termite commonly found in decayed oak trees on the University campus. The flagellate infests the posterior and midregions of the intestinal tract of the termite with only a slight infection or none at all of the anterior region. It is found in the lumen of the canal with no attachment to the wall. Associated with it is a large *Trichonympha*, and in cross-section of the entire intestinal tract it is found that these two flagellates, with the latter usually predominating, completely fill the lumen of the canal.

Almost every individual of this species of termite which has been examined has been found to harbor these flagellates. The number in a single host may vary greatly as it or the trichonymph may be the dominant form. In some instances the latter species may be rare with *Trichomitus termitidis* present in vast quantities. These are sporadic cases, however, with no indications of a rhythmical cycle

that is seasonal in its occurrence, as shown by examinations of the host which have been made throughout the year.

Trichomitus greatly resembles its near relative *Trichomonas* in its activities. It is, however, difficult to keep these flagellates alive in cultures, hence observations on the active forms have been limited. The extreme fragility of the cytoplasmic body as contrasted with the stout, persistent parabasal body, is particularly striking in preparations of living material. A few seconds usually suffices, in ordinary tap water, for the dissolution of the protoplasm, leaving the neuromotor system still intact.

Nutrition in *Trichomitus* is holozoic. It, like *Trichonympha*, is evidently only a commensal, or at least is not truly parasitic, i.e., living on the tissues or fluids of the host. The food particles found within the cytoplasm consist principally of woody fibers upon which the termite feeds.

MORPHOLOGY

The morphology of this species of *Trichomitus* is of especial significance not only in view of the distinctness with which the neuromotor organ system is developed and integrated but also in the unquestionable certainty with which the relationship of the centrosome to the blepharoplast is established, as will be shown later. The relatively large size of the organisms (75 to 150μ) and their abundance have made possible an analysis of these structures not obtainable with the smaller trichomonads of our earlier studies (Kofoid and Swezy, 1915).

Shape and Size of Body

The body of *Trichomitus termitidis* is exceedingly amoeboid and protean in life, having neither constancy of form nor resistance to deformation on contact with other organisms or objects. Its periplast is unusually thin and delicate and in the larger forms especially is not infrequently ruptured in the making of smear preparations. It has nevertheless a certain characteristic range of forms within which, in free movement, it is seen or preserved on fixation. These vary from the asymmetrical pyriform contour, with the large end anterior and the posterior tapering to a blind point (pl. 3, fig. 1), to the ellipsoidal (pl. 3, fig. 14) or subspheroidal shape (pl. 3, fig. 5), with the slightly greater diameter posterior to the center.

The factors conducing to these changes in form are the stages of general contraction of the body, the mass of food vacuoles which is usually greater in the more rounded forms, and the proportional length of the parabasal body and undulating membrane. As a result of multiple fission (pl. 4, figs. 28, 30) one of the daughters receives the ancestral parabasal and two of its associated flagella, both of which are disproportionally large for the cytoplasmic mass of this schizont. Regulative resorbtion, in (pl. 4, fig. 31) or out of a cyst, or rapid cytoplasmic growth, would be necessary to readjust the volumetric relation of the neuromotor system and the cytoplasmic mass.

The range in size in this species is very considerable (fig. A, 1–7). The smallest schizonts we have recorded (pl. 4, fig. 32) are but 16μ in length while the largest exceed 200μ. These giant individuals are probably approaching multiple fission. They rarely survive the smearing operation intact. Not infrequently the nucleus and its attached neuromotor system of such giant individuals will be found intact and still active after the loss of the cytoplasm indicating that this stage is particularly susceptible to destruction under normal conditions in the host. Most of the individuals seen range from 75 to 125μ in length.

The organs of *Trichomitus* (fig. A, 5) consist of the cytostome (*cyt.*), nucleus (*n.*), food vacuoles (*f. vac.*), and the neuromotor organ system. We will now consider these organs with the cytoplasm in detail.

Cytoplasm

The cytoplasm of *Trichomitus termitidis* is reasonably labile, finely granular, and somewhat alveolar in structure. This lability may be the cause of such abnormal proportions (such as are seen in plate 4, figure 30) rather than the inheritance of the ancestral parabasal suggested above. The dropping off by plasmotomy of the labile cytoplasm has been observed by us in *Trichomonas augusta* (Kofoid and Swezy, 1915). No contractile vacuole is present but food vacuoles (fig. A, 5, *f. vac.*) are found everywhere within the body except about the nucleus. These contain fragments of cellulose from the digestive tract of the termite or coccoid bodies, possibly bacterial (pl. 3, fig. 5). Defecation of undigested fragments has not been seen.

Upon treatment with neutral red a large number of food particles or metaplasmic droplets stain deeply. They lie scattered throughout the cytoplasm and are larger near the center of the body (fig. A, 8).

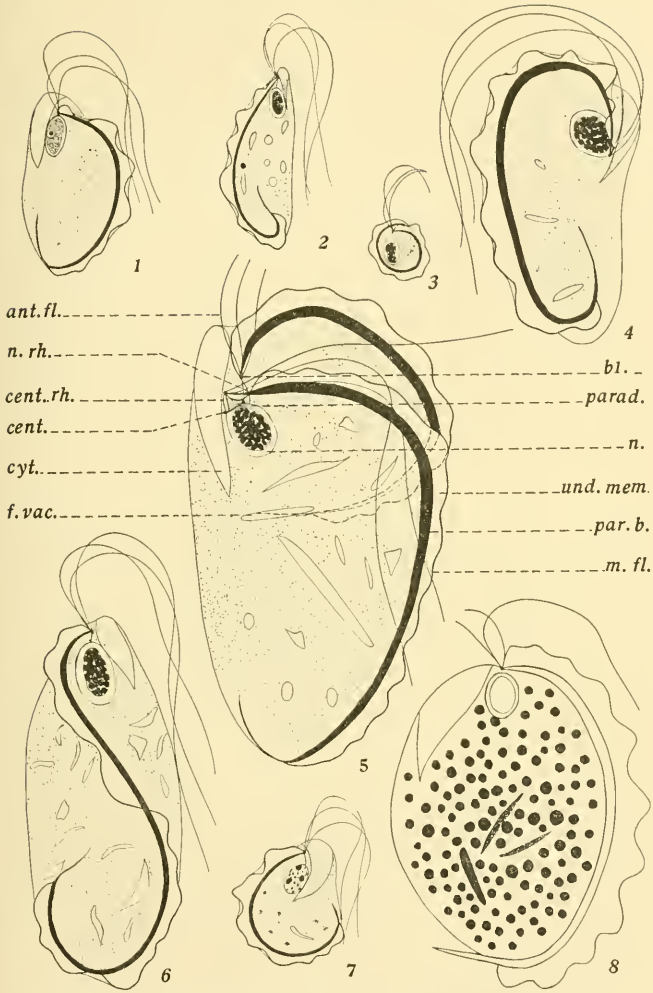

Fig. A. A series showing variations in size in *Trichomitus termitidis*. Figure 8 is drawn from individual stained with neutral red.

Abbreviations: *ant. fl.*, anteror flagella; *bl.*, blepharoplast; *cent.*, centrosome; *cent. rh.*, centrosome-rhizoplast; *cyt.*, cytostome; *f. vac.*, food vacuole; *m. fl.*, marginal flagellum; *n.*, nucleus; *n. rh.*, nuclear-rhizoplast; *par. b.*, parabasal body; *parad.*, paradesmose; *und. mem.*, undulating membrane. × 700.

Cytostome

The cytostome lies on the anterior ventral surface at the extreme anterior end of the body. It is a large elongated asymmetrical pocket, slender pyriform in outline but curved on its right side against the nucleus. Its length is about 0.3 that of the body and its width 0.3 to 0.2 its own length. It leads into the cytoplasm near the center of the body. Its large size, great flexibility and its slight projection anteriorly in a prominent lip all indicate its efficiency as a food-grasping and enveloping organ. We have found it during the later stages of binary fission, in the plasmodium of multiple fission, but not in the encysted condition. We have not been able to determine the exact mode of origin of new cytosomes. Its location immediately adjacent to the blepharoplast and nucleus necessitates a high degree of elasticity, integrity and resistance on the part of these organelles and the rhizoplasts arising from them.

Neuromotor System

The use of the term organ system to designate the complex, structurally integrated apparatus which links together the nucleus and motor organs and plays such a distinctive rôle at mitosis, seems justified by the canons of comparative morphology, unless it be that the dogma of the Cell Theory blights such morphological license. This organ system includes the blepharoplast (fig. A, 5, *bl.*) from which spring directly the three anterior flagella (*ant. fl.*), the attached, posteriorly directed undulating membrane (*und. mem.*) with its marginal flagellum (*m. fl.*), and the deeper lying parabasal body (*par. b.*), and a nuclear rhizoplast (*n. rh.*). The centrosome (*cent.*) lies within the centroblepharoplast, emerging at mitosis with its own independent centrosomal rhizoplasts (*cent. rh.*) joining the ends of the paradesmose (*parad.*) to the parent blepharoplast.

It is noteworthy from the standpoint of comparative cytology that the motor organelles, flagella, and undulating membrane terminate in and originate from the centroblepharoplast. The nucleus never loses its connection, by one or more rhizoplasts, with this structure, which also plays a dominant rôle in the drama of mitosis for at this time there springs from it the centrosome, which later divides, forming the paradesmose and its connecting rhizoplasts. To it also is attached the enormously large parabasal body, a reservoir of chromatoidal sub-

stance. This centroblepharoplast is thus most truly a morphological center intimately associated with the motor organs of this cell and their activities during the vegetative phase. At mitosis it dominates not only the extra-nuclear neuromotor system but also the polarization and subsequent movements of the chromosomes within the nucleus (fig. B, and pl. 3, figs. 5–21) as well. It will be difficult to find in any metazoan cell so continuous and complete a control, so pervasive an influence upon cell activities by the cell organ there known as the centrosome, as we find by the centroblepharoplast in *Trichomitus*.

The *centroblepharoplast*, during the vegetative phase of *Trichomitus* (fig. A, 6), is a minute granule about a micron in diameter anterior to the nucleus and attached to the anterior end of a single nuclear rhizoplast (fig. A, 5, *n. rh.*; fig. A, 2). This rhizoplast is a delicate thread easily overlooked. The centroblepharoplast itself is imbedded in the end of the deeply staining parabasal body, and may likewise readily escape detection.

In view of its later history it seems advisable to designate this granule at this period as the centroblepharoplast, since from it emerges the parent centrosome at mitosis. There is, however, no duplicity of structure evident, and there is no granule at any time at the point where the nuclear rhizoplast passing from the centroblepharoplast (fig. B, 1) and later from the blepharoplast proper (fig. B, 5) meets the nuclear membrane. After the centrosome withdraws from the larger granule (fig. B; pl. 3, figs. 6–21), the latter becomes a blepharoplast in the restricted sense of a basal granule from which the flagella originate, having no other function in mitosis, whereas the centrosome emerging from it divides and its daughters form the paradesmose between them, assume a polar position thereon and move to the nucleus (fig. B; pl. 3, fig. 21).

In our investigations of mitosis in the trichomonads (1915, pl. 2, figs. 21, 23; pl. 3, figs. 24, 29) there appeared to be a separation of the polar centrosome-blepharoplast into two granules, one of which, the centrosome, remained in the polar position on the nucleus, and the other, the blepharoplast, usually with the flagella attached, was removed a short distance therefrom. The conditions which we have found in *Trichomitus* where there is a general, more complete and perfectly distinct separation of these two organelles is thus the full accomplishment of the segregation imperfectly realized in *Trichomonas*.

The *flagella* are four in number, the three undifferentiated, equal, anterior ones (fig. A, 5, *ant. fl.*) and the attached posteriorly directed

one included within the undulating membrane as its marginal fiber (*m. fl.*) and carried out beyond the projecting tip of the parabasal body as a bit of free flagellum. The anterior flagella usually exceed the body in length. The posteriorly directed location in our figures is merely for spatial accommodation, an anterior direction being usual in life.

The *undulating membrane* (fig. A, 5, *und. mem.*) is attached to the left side of the body (fig. A, 1) in a sweeping C- or S-shaped curve reaching to the posterior end of the body (fig. A, 4, 6; pl. 3, figs. 1, 14). It exceeds the length of the body two to three times in some small schizonts (pl. 4, fig. 30). The coiling into the S-shaped forms appears to be an accommodation of the somewhat rigid but elastic parabasal, when longer than the body, to its location within the cytoplasm. The membrane always follows the course of the parabasal and remains adherent to it upon cytolysis (pl. 3, fig. 11). In one case (pl. 3, fig. 4) a detached membrane consisting only of the marginal flagellum and the fold of the protoplasmic pellicle running from the parabasal around the flagellum, was found free in a smear preparation. The membrane and flagellum are thrown into twelve to twenty subequal, subequidistant waves of contraction which fade out in the distalmost end.

The parabasal body (fig. A, 5, *par. b.*) is a rigid, elastic, deeply staining, chromatoidal rod lying at the base of the undulating membrane in the peripheral plasma of the body. Its C- or S-shaped course appears to determine the direction of that membrane. Its length usually exceeds that of the body by 10 to 25% and its diameter, 2 to 3.5μ, is greatest somewhat anterior to its middle. From this region it tapers gradually toward either end, terminating anteriorly at the centroblepharoplast (fig. A, 6), or in mitosis at the blepharoplast proper (fig. A, 5), and posteriorly at its junction with the marginal flagellum which projects beyond their union for a short distance as a free lash. It stains densely with haematoxylin and constitutes the dominating feature of the organism in all preparations and in life. It shows in stained sections (pl. 3, figs. 3 and 3*a*) a differentiated structure consisting of an outer deeply staining shell less than a fifth of its diameter in thickness and a less deeply stained core. This core is traversed by wedgelike discs of the cortical substance, which arise principally on the concave face and fade away towards the opposite side.

We have elsewhere (Kofoid and Swezy, 1915; Kofoid, 1917) interpreted the parabasal body as a reservoir of substances utilized by the neuromotor system in motor activities. It is obvious on observation

that locomotion by *Trichomitus* in the midst of the seething mass of parasites in the digestive tract of *Termopsis*, involves not a little expenditure of energy. It is also conceivable that the conditions of life therein are subject to marked variations incident not only to the food and feeding of the host but also to the varying constituents of the enormous mass of parasitic associates and their changing metabolism due to phases of their reproductive activity. Biochemical changes of no small import are consequently a feature of this creature's environment. That some of these are peculiarly fatal to *Trichomitus* is evident from the unusual numbers of moribund or cytolized individuals, each represented by a more or less decadent nucleus and its attached neuromotor apparatus, which may be found in most smear preparations.

Considerable changes in extent and volume of this structure are apparent upon an inspection of our figures, and even more so in our preparations. These are indicative of changes resulting from metabolism, or multiple fission, or both. In addition to the storage or reservoir function it is apparent that the parabasal in *Trichomitus* serves also as a somewhat rigid organ of attachment for the undulating membrane.

The other organs of the neuromotor system, the paradesmose and its rhizoplasts will be discussed in connection with mitosis. The paradesmose is a more or less temporary organ in most trichomonads, but in *Trichomitus* the organism appears to pass a much greater part of its existence in what is comparable to the prophase stage of trichomonad mitosis, so that the paradesmose is actually present, suspended by rhizoplasts from the blepharoplasts (fig. A, 5), and the whole neuromotor system is in some phase of duplication in many of the individuals which we have seen. The stage with a single centroblepharoplast and rhizoplast (fig. A, 1, 2, 6) is relatively much less common in this species than in other trichomonads. This prolongation of the prophase is the first step towards diplozoic organization such as we find realized in *Giardia*, where nuclear division is added to that of the duplication of the neuromotor system with the resulting formation of a coördinating system for multicellular organization.

Nucleus

The nucleus (fig. A, *n.*) is a symmetrical ellipsoidal structure, or even ovoidal or pyriform, with the wider end posterior (fig. B, 1). The longer axis is two to three times the shorter one in length. It

lies in or near and parallel to the major axis of the body on the left side of the cytostome within a short distance of the anterior end of the body. It shows distinctly a peripheral clear zone which is somewhat regularly chambered (fig. A, 1, 4, 5; pl. 3, fig. 9) as we have found it also in *Trichonympha*. This zone surrounds the dark, dense and often seemingly undifferentiated central chromatin mass. On heavy destaining this central mass is at times resolved into fairly uniform rounded granules (pl. 3, fig. 2) which appear to have some special relation to the elements of the chambered zone, indicating the possibility of a persistent organization of the nucleus. In some instances resting nuclei (fig. B, 1; pl. 3, fig. 5) show large deeply staining granules resembling nucleoli but these are as a rule absent. It is quite possible that these may be end knobs of emerging chromosomes.

The size of the nucleus ranges from 10 to 20μ in length whereas that of the body ranges from 16 to 200μ or even more. Although larger individuals have larger nuclei the increase in the volume of the cytoplasm is many-fold greater than is the increase of the nucleus in these giant forms.

One of the most significant and striking features of the life history of *Trichomitus* in the digestive tract of *Termopsis* is the repeated and seemingly constant occurrence of large numbers of isolated neuromotor systems with the nucleus attached but no enveloping cytoplasm. It is evident that the delicate pellicle is easily destroyed, and the labile cytoplasm escapes. Such an isolated structure in late prophase with duplicated neuromotor systems but degenerate nucleus is seen in plate 3, figure 11. These occurrences afford indisputable evidence of the organic continuity and structural integration of the neuromotor system of *Trichomitus* and of its direct and efficient physical connection with the nucleus. One of us (Swezy, 1915*a*) has noted a similar phenomenon in *Hexamitus*, a diplozooic polymastigote.

Still more significant is the fact that such isolated systems are still capable of flagellar activity and locomotion after the destructive process of cytolysis of the cytoplasm. They continue to move for some time in smears of the contents of the digestive tract mounted in tap water. The very large number of isolated systems found in some smears is indicative of a considerable period of persistence of the isolated neuromotor system and nucleus after the loss of cytoplasm. It is obvious that grave limitations on such activities must arise as a result of the loss of the cytoplasm. The nucleo-cytoplasmic reactions are suspended, nutrition is impeded if not wholly suspended, and rapid exhaustion is accelerated by the loss.

MITOSIS

Owing to this prolongation of the prophase of mitosis in *Trichomitus termitidis* an exceptional opportunity is afforded for a detailed study of the behavior of the neuromotor system during mitosis. This is made possible by reason of the fact that the centroblepharoplast is the center of the neuromotor system and the point of origin of structures and processes playing the main rôle in mitosis.

The phases of mitosis recognizable in the division of *Trichomitus* are those of the metazoan cell, but, as shown in our (1915) discussion on mitosis in trichomonads, considerably differentiated by the association of the extra-nuclear organelles of the cell in the protozoan from that in the usually simpler metazoan unit. In *Trichomitus* these differences, in consequence of the massive development of the neuromotor system, are even more developed than in the other trichomonads. They consist mainly in the sharp separation of centrosome and blepharoplast and the excessive prolongation of the prophase.

The *resting stage* of *Trichomitus* has a single nuclear rhizoplast running from the centroblepharoplast to the anterior end of the nucleus where it is attached to the membrane without evidence of enlargement into a centrosome on the nuclear membrane. This rhizoplast is often very short (fig. A, 1) and is never very long. The nucleus has in this period a dense, coarsely and uniformly granular central chromatin mass in which no polarization or evidence of chromosome formation is present.

The *prophase* (fig. B; pl. 3, figs. 1, 2, 5–15) is a prolonged one and in it the duplication of the entire neuromotor system takes place by division and outgrowth from the centroblepharoplast. The initial step is the splitting of the centroblepharoplast and the nuclear rhizoplast (fig. B, 1). It appears in some cases (pl. 3, fig. 1) that the rhizoplast may divide at the nuclear membrane first and split distally towards the centroblepharoplast as though these were directly upon the nuclear membrane at the anterior end of the nucleus. At the same time the centroblepharoplast separates into its constituent centrosome and blepharoplast, with the latter immediately dividing, one granule taking a single flagellum, the new parabasal body and one of the two nuclear rhizoplasts (fig. B, 1). The granule remaining attached to the old parabasal body takes the remainder of the flagella and the second nuclear rhizoplast. As these two blepharoplasts separate a thread is drawn out from each attaching them to the centro-

some (fig. B, 1). This is followed by a division of the centrosome. As the two new centrosomes move apart a darkly staining line or bar is drawn out between them, the paradesmose (fig. A, 5, *parad.*; fig. B).

The rhizoplasts connecting the centrosomes with the blepharoplasts gradually elongate with the paradesmose also increasing in both length and thickness. The latter structure with its connected centrosomes moves down until it comes to rest upon the nuclear membrane (fig. B). As a result of this the centrosome-rhizoplasts come to have the same length as the nuclear rhizoplasts. All four of these rhizoplasts are exceedingly delicate, particularly the nuclear ones and the latter especially long escaped our notice. Since the two are rather close together (fig. B, 5) their distinctiveness may be easily overlooked. The centrosomes (fig. A, 5, *cent.*) are minute knobs on the ends of the paradesmose, which is a stout, heavy, sometimes granular, deeply staining bar. They are not always visible as expansions of the bar and are never seen detached from it.

In the meantime the new undulating membrane and parabasal body have reached sizes equal to the ancestral ones (pl. 3, fig. 11; fig. B, 2–6). The parabasal body first appears as a slender dark thread growing posteriorly subparallel to the old parabasal in the peripheral cytoplasm (pl. 3, fig. 5). With the beginning of the formation of the paradesmose (fig. B, 2) the new flagella have all formed by outgrowth from the daughter blepharoplasts, two anterior ones and a posteriorly directed one as a marginal filament from the blepharoplast attached to the new parabasal, and only one anterior one from that attached to the old parabasal. At first (fig. B, 2) the new undulating membrane is very narrow but it soon attains full structural size and functional efficiency (pl. 3, fig. 11). With the completion of these structures by growth the duplication of the neuromotor system is accomplished.

Up to this time the changes visible within the nuclear membrane have been very slight. The chromatin granules or chromomeres grow larger and darker, and evidences of polarization appear in the linear grouping of the granules (fig. B, 6; pl. 3, figs. 6–10) which culminates in the emergence of linear V-shaped chromosomes. During this process a deeply staining cone-shaped extension of the central chromatin mass projects anteriorly until it comes in contact with the paradesmose (fig. B, 6; pl. 3, figs. 6–8). As this disappears the V-shaped chromosomes become more evident in the central mass, and

gradually spread out below the paradesmose as though hung across a string suspended from its ends (pl. 3, figs. 10, 12, 13).

The number of chromosomes is rather obscure since the loops are at all times rather closely entangled. It appears to be twelve or thereabouts. In the earlier phases each is composed (pl. 3, fig. 10) of a line of distinct granules, like chromomeres, but these fade out as the metaphase approaches.

Fig. B. Development of paradesmose in *Trichomitus termitidis*. 1. Outgrowth of new parabasal body after separation of centrosome and blepharoplast and division of latter. 2. Centrosome divided and paradesmose forming between them; new flagella and undulating membrane formed. 3. Elongation of paradesmose as it moves down to the nucleus with the lengthening of the centrosome-rhizoplasts. 4. Later stage of same, condensing chromatin in nucleus with cone-shaped projection starting towards the nuclear membrane and paradesmose. 5. Paradesmose attached to nuclear membrane. 6. Attachment of central chromatin mass to paradesmose and formation of spindle; beginning of formation of chromosomes. × 1575.

The *metaphase* is obscured by the fact that there appears to be no arrangement of the chromosomes in an equatorial plate and no amphiaster phase of the nucleus. The nuclear structures appear to conform their arrangement to the stout bar-shaped paradesmose and not the latter mould itself to the configuration of the nucleus as in *Trichomonas* and *Eutrichomastix* (Kofoid and Swezy, 1915).

The loops or V-shaped chromosomes are gradually drawn by the angle of the V towards the two ends of the paradesmose (pl. 3, figs. 15, 17, 18). It is possible that each original loop is split lengthwise during this movement but the evidence for it is by no means clear. During this process the loops shorten, thicken, and stain more deeply, so that when they have finally parted (pl. 3, figs. 16, 20, 21) they form chrysanthemum-shaped rosettes.

The *anaphase* is brief and is also dominated by the stout paradesmose, which continues to produce a one-sided, asymmetrical grouping of the two groups of parting chromosomes and to modify the constriction of the nuclear membrane so that it is also one-sided. The nuclear membrane remains intact throughout the whole process of mitosis. In the late anaphase constriction is completed, the nuclei separate and move apart (pl. 4, fig. 22), stretching out the paradesmose between them as a result of the uncoördinated activities of the two daughter neuromotor systems, which are attached to the nuclei by their rhizoplasts.

The *telophase* (pl. 4, fig. 36) ensues before plasmotomy. In it the chromatin of the massed chromosomes rounds up in the central mass and the clear zone reappears and the ellipsoidal form is resumed. The paradesmose also fades away, and the centrosome merges with the daughter blepharoplast which, by the shortening of the nuclear rhizoplast, comes to lie closer to the nuclear membrane, thus bringing the schizont back to the nuclear condition prior to mitosis.

The process of mitosis in *Trichomitus* is similar to that in other trichomonads in that the nuclear membrane remains intact throughout the process, the extra-nuclear paradesmose arises between the daughter centrosomes, and the duplication of the neuromotor system proceeds from the centroblepharoplast and takes place prior to division of the chromosomes. It differs in having a distinct separation of centrosome and blepharoplast for a long period, in having a rigid bar-shaped paradesmose, and in the large size and greater elongation of the chromosomes. This higher specialization is conditioned by crowded conditions of parasitic life in association with other parasites of relatively complex organization. The conditions of locomotion in this association and the excessive amount of stimulation consequent thereon are causes conducive to the extraordinary development of the neuromotor apparatus in *Trichomitus* and the resulting modifications in mitosis.

BINARY AND MULTIPLE FISSION

Both of these processes take place frequently in *Trichomitus*. There is much evidence of a high death rate in this species within the digestive tract of its host. This is compensated for by rapid multiplication. For this also there is abundant evidence in our material.

The distinction between stages of binary and multiple fission is not readily made in all cases in early stages. The earliest phases of both are obviously the same in mitotic phenomenon. Binucleate plasmodia may lead on to further division when prophases appear in their nuclei (pl. 4, fig. 23). When, however, no later prophase phenomena are evident and the organism is not unusually large (pl. 4, fig. 36) binary fission only may be expected. It also occurs in the small cysts (pl. 4, fig. 26) and small free forms (pl. 4, fig. 29).

Multiple fission, on the other hand, occurs in large individuals and leads to the formation of, presumably, eight-celled plasmodia.

One such large plasmodium with six constituent zooids is seen in plate 4, fig. 28. It is possibly in plasmotomy and has lost two of its members. Not all multiple fission plasmodia are as large as this. Smaller ones, in which the first division has been completed and the second initiated, are frequently found (pl. 4, figs. 23, 25, 35).

The process is one of three repeated divisions prior to plasmotomy with the formation of an eight-nucleate somatella and its subsequent disintegration into its constituent zooids by their detachment singly or in groups. These somatella or plasmodial stages are exceedingly mobile and the constituent individuals shift about without seeming order of arrangement. The uncoördinated movements of the powerful neuromotor apparatus of the individual zooids finally result in their separation. The connecting paradesmoses are lost long before this separation.

No trace of unequivocal sexual phenomena has been detected. Large and small individuals simulating macrogametes and microgametes and gametocytes are present. Binucleate individuals without evidence of recent division occur (pl. 4, fig. 36), simulating zygotes, and similar associations are found in cysts (pl. 4, fig. 26). There is, however, no evidence of maturation divisions leading to gamete formation, no sexual behavior detected, and no evidence of the fusion of gametic nuclei. In the absence of such evidence any conclusions as to the possibility of sexual reproduction in this organism must be held in abeyance.

ENCYSTMENT

Associated with the vegetative and fission stages of *Trichomitus* in a few hosts but not all, we have found many small individuals (pl. 4, figs. 24, 29–33) in which binary fission is occurring and in which there is a tendency for the body to round up into a spheroidal or ellipsoidal mass. These small sizes may result from rapid fission without compensating growth or from plasmotomy of part of the cytoplasm. In these same hosts occur also numerous ellipsoidal cysts about 13 by 20μ with a deeply stained network with thickenings at the nodes spread over the surface (pl. 4, figs. 26, 27). The cyst wall is double and the network is due to the accumulations of some stainable substance between the walls. Within the cyst is a single individual (pl. 4, fig. 31) with a very long parabasal and undulating membrane making nearly two complete coils, such as might result from the coiling up of an individual with abnormally large neuromotor system (pl. 4, fig. 30). In other cases two such individuals (pl. 4, fig. 26) are found within the cyst. This might result from encystment after or during mitosis but prior to plasmotomy. Such cysts may facilitate the carrying over of infection from one individual host to another. They have all the indications of being resistant stages.

RELATIONSHIPS

This is a species of *Trichomitus*, a genus founded by one of us (Swezy, 1915*b*) for the reception of a minute and simple trichomonad from amphibians. In its vegetative phases the form here described has the morphological features of *Trichomitus parvus* Swezy, namely, three anterior flagella, undulating membrane, parabasal, and no axostyle. It differs greatly in size, in the massive development of the parabasal, and at mitosis in the distinct separation of centrosome and blepharoplast. Binary and multiple fission were followed in the species from amphibians but no trace of such separation was detected.

Such a difference as this might justify generic separation but it might be impracticable to apply it in future diagnosis of any species of the genus which may come to light. The difference is, however, of such morphological import as to justify subgeneric separation. We accordingly assign it to

Trichomitopsis subgen. nov.

Trichomitus with centrosome separated from blepharoplast at mitosis. Type species *Trichomitus termitidis* sp. nov. from *Termopsis angusticollis* Walker.

SUMMARY

1. *Trichomitus termitidis* sp. nov. occurs in the intestinal tract of *Termopsis angusticollis*. It is apparently not pathological to its host, is never attached to the wall and feeds on the débris of the intestinal contents.

2. It has a highly developed neuromotor system with parabasal body, undulating membrane, centroblepharoplast and flagella attached by a rhizoplast to the nucleus.

3. Binary fission occurs frequently. Mitosis is marked by the development of a large paradesmose following the separation of the centrosome from the blepharoplast. One schizont retains the old parabasal body and membrane, while new ones are formed for the other.

4. Multiple fission results in the formation of an eight-zooid somatella followed by plasmotomy.

5. Owing to the great differences in the process of mitosis between *Trichomitus parvus* and the new species, *T. termitidis*, subgeneric distinction is given to the latter, as we assign it to the new subgenus *Trichomitopsis*.

Zoological Laboratory, University of California,
Berkeley, California.
Transmitted September 6, 1918.

LITERATURE CITED

Kofoid, C. A.
1917. The biological and medical significance of the intestinal flagellates. Proc. Second Pan Amer. Sci. Cong., 1915–16, **10**, 546–565.

Kofoid, C. A., and Christiansen, E. B.
1915. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–54, pls. 5–8, 1 fig. in text.

Kofoid, C. A., and Swezy, O.
1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad. Arts Sci., **51**, 289–378, pls. 1–8, 7 figs. in text.

Swezy, O.
1915*a*. Binary and multiple fission in *Hexamitus*. Univ. Calif. Publ. Zool., **16**, 71–88, pls. 9–11.
1915*b*. On a new trichomonad flagellate, *Trichomitus parvus*, from the intestine of amphibians. *Ibid.*, **16**, 89–94, pl. 12.

EXPLANATION OF PLATES

All figures of *Trichomitus termitidis* sp. nov., from material stained with iron alum haematoxylin. Magnification 625, unless otherwise stated.

PLATE 3

Fig. 1. Trophozoite in early prophase of division with nuclear rhizoplast divided.

Fig. 2. Isolated neuromotor apparatus of the same stage with nucleus attached.

Fig. 3. Sagittal section of the parabasal showing the outer deeply staining shell and the inner core. × 1250.

Fig. 3*a*. Cross-section of the same. × 1250.

Fig. 4. Isolated undulating membrane with darkly staining marginal flagellum. × 1250.

Fig. 5. Early prophase with outgrowing of new parabasal body.

Fig. 6–10. Early prophase stages showing gradual condensation of chromatin into definite chromosomes. New parabasals, undulating membranes and flagella complete.

Fig. 11. Isolated neuromotor system in prophase stage of division.

Figs. 12, 13. Spindle forming with chromosomes attached to it by the angle of the V. × 1250.

Fig. 14. Prophase. Note relative lengths of parabasal bodies.

Figs. 15, 17. Separation of chromosomes into two groups. × 1250.

Figs. 16, 18–20. Anaphase with paradesmose elongating. × 1250.

Fig. 21. Beginning of constriction of the nuclear membrane. × 1250.

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PLATE 4

Fig. 22. Late anaphase, showing formation of new cytostomes. Note length of paradesmose.

Fig. 23. Multiple fission with beginning of the third division of nucleus.

Fig. 24. Small individual apparently about to encyst.

Fig. 25. Multiple fission with nuclei in preparation for second division.

Fig. 26. Encysted form which has divided. Note structure of cyst wall. × 1250.

Fig. 27. Encysted form showing outer surface of cyst only. × 1250.

Fig. 28. Somatella of six zooids formed by multiple fission. Full complement of flagella, parabasals, undulating membranes and nuclei.

Fig. 29. Dividing individuals just escaped from cyst.

Fig. 30. Product of multiple fission. Note relatively enormous length of parabasal.

Fig. 31. Encysted form with single nucleus.

Fig. 32. Individnal following excystment.

Fig. 33. Small form dividing. Note relative lengths of parabasals.

Fig. 34. Late anaphase of binary fission.

Fig. 35. Second division of nucleus in multiple fission.

Fig. 36. Telophase of binary fission.

22
23
25
27
28
31
32
34

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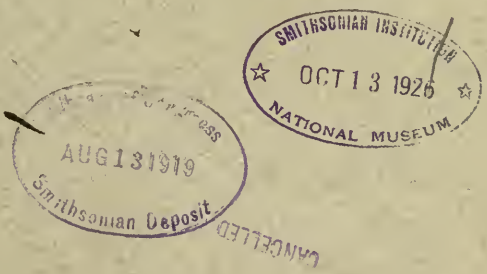
BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY



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Smithsonian Deposit

CONTENTS

PAGE

Introduction 42
Occurrence and activities 43
Morphology 44
Shape and size 45
Neuromotor system 47
Ectoplasmic structures 49
Surface ridges 49
Locomotor organelles 50
Oblique fibers 52
Centroblepharoplast 53
Alveolar layer 54
Transverse myonemes 55
Endoplasmic structures 56
Longitudinal myonemes 57
Nucleus 57
General discussion 59
Binary fission 61
Division of the neuromotor apparatus and ectoplasmic structures 62
Mitosis 65
Prophase 65
Metaphase 68
Anaphase 68
Telophase 69
Discussion of mitosis 69
Relationships 77
Summary 81
Literature cited 82
Explanation of plates 84

INTRODUCTION

Among the instances of parasitism by the Protozoa in the alimentary tracts of the Metazoa, none exceeds that found in the termites as to the relative volume of the invading parasites, the diversity of organization found among them, and the degree of specialization which they attain. In the vanguard of this evolutionary development stands the genus *Trichonympha* discovered by that pioneer American investigator, Dr. Joseph Leidy, who in 1877 and 1881 first revealed this teeming menagerie of termite parasites to scientific view.

The extent of specialization attained by *Trichonympha* and its allies has obscured their relationships, allied them at first with the ciliates, and obliterated their true affinities with the flagellates. The purpose of the present paper is in part to place the flagellate origin and relationships of the Trichonymphidae on a firm cytological foundation. It is also our aim to set forth the most complicated neuromotor system thus far known among the Protozoa, to analyse its elements, relate them to the elaborate motor activities of the individual, and trace their behavior during the mitosis of this highly specialized cell.

The degree of structural complexity, the extent of the coördinated mechanism, the number of its constituent elements, and the striking similarity of their interrelations in *Trichonympha* to those obtaining in multicellular organisms, is illuminating as to the biological significance of cellular organization. This organism is a single cell, with one nucleus, yet it has attained a degree of structural complexity and functional diversity in respect to one organ system comparable almost with that of its host and surpassing that of many of the lower Metazoa. The multicellular state is plainly not an essential condition for evolutionary specialization and functional efficiency, except as it places limits on the size of organisms and on developmental processes arising therefrom. The differentiation of organs within the confines of a single cell is here accomplished with a perfection comparable with that where the organ is a complex of diverse cells instead of one of many parts within a single cell. The organism and not its cells is the fundamental basis of differentiation.

OCCURRENCE AND ACTIVITIES

The posterior and mid-regions of the intestinal tract of *Termopsis angusticollis* Walker are usually found to be greatly distended, often filling the entire cavity of the body. It is in these regions that *Trichonympha campanula* is found in great abundance, filling the entire lumen of the canal but never attached to its walls. When other flagellates, as *Streblomastix strix* and two other smaller forms, are present with it in any numbers, these are found occupying the region near the walls, with *Trichonympha* filling the central portion of the canal.

The intestinal contents resemble a thick milky fluid, the great consistency of which is due to the vast numbers of protozoans which it contains, along with minute débris of woody particles. Through this mass the trichonymphs move with considerable rapidity, using the mobile anterior portion of the body to clear a pathway. This is done by quick sidewise movements, such as those shown in figure C, 2, bending the anterior cone first to one side and then to the other without halting in its progress. Its path is usually straight ahead with little or no rotation of the body on its longitudinal axis.

The group of long anterior flagella seems to be its main propelling organelle. These are thrown forward and sidewise somewhat as other flagellates use their flagella, with, however, less of the forward motion than is usual with anteriorly attached flagella. The flagella or cilia on the remainder of the body are uniformly directed posteriorly. These are found to have a characteristic motion, both during locomotion of the organism and when it is at "rest." Waves of contraction pass constantly from the anterior end backward to the tips of the flagella, affecting all on any given plane alike. The motion of the group of longer, anterior flagella, particularly when they are directed posteriorly, as is frequently the case, may coincide with these waves of contraction, which then include all the ciliary covering of the body. These waves continue during observation on the slide until the rounding up of the body in the culture fluid prior to dissolution.

These flagellates are extremely susceptible to environmental changes. When placed in culture slides with tap water, distilled water, normal salt solution, or Ringer's fluid, the results have been disastrous in a very short time, varying from a few minutes to half an hour. The body rounds up with considerable distension, becomes transparent with an almost complete loss of organelles, the nucleus only remaining visible until the final dissolution of the body.

The flagella continue moving as long as they are visible at the beginning of this process but soon disappear.

Dilute egg albumen has been found to be the most satisfactory culture medium but even with this these flagellates have been kept alive only a few hours, with the rounding-up process soon visible in a majority of the forms.

As has been earlier noted for trichonomad flagellates (Kofoid and Swezy, 1915), binary fission in *Trichonympha campanula* is cyclic in its occurrence, being found abundantly in occasional individual hosts.

In order to secure all stages of the division cycle, preparations were made every day continuously for thirty days, the number of individual hosts used each time varying from three to ten or more. For several days also these preparations were made at two-hour intervals during the day. In this way we have been able to secure a fairly complete series of figures of all stages of the mitotic process. A few isolated division forms may be present in almost any host, but in general it has been found that where more than these occur in a single host, that from one-third to one-half or even more of the individuals of a single parasitic species observed, will present some signs of division and usually of a single stage of mitosis. This is especially true of the early prophase stages which, on the whole, have been most abundant in our material. A few slides have been found, however, on which almost all stages of the division process may be found. This seems to be the exception and not the rule.

MORPHOLOGY

Every observer of the parasites of the digestive tract of the termites is amazed, on his first glance at its seething contents, at the locomotor activities displayed by its constituent organisms. Foremost among these in constancy, agility, and variety of its movements is *Trichonympha*. It is significant that Leidy (1877) used the specific name *agilis* for the first trichonymph discovered. This capacity for motor activities is based on structural features of corresponding complexity. Hence it is that any discussion of the morphology of this animal is mainly occupied with the neuromotor system. The only other differentiated structure found in the body is the nucleus. There is no apparent mouth, no excretory system, no food-taking organs. The food vacuoles are temporary in a seemingly undifferentiated endoplasm. The structural specialization of this parasite thus affects mainly one organ system only, the neuromotor system.

Shape and Size

The shape of the body is companulate with the posterior end broadly rounded (fig. B), almost spheroidal, while anteriorly it is a tapering, slightly convex cone of 20°. The anterior half of the body

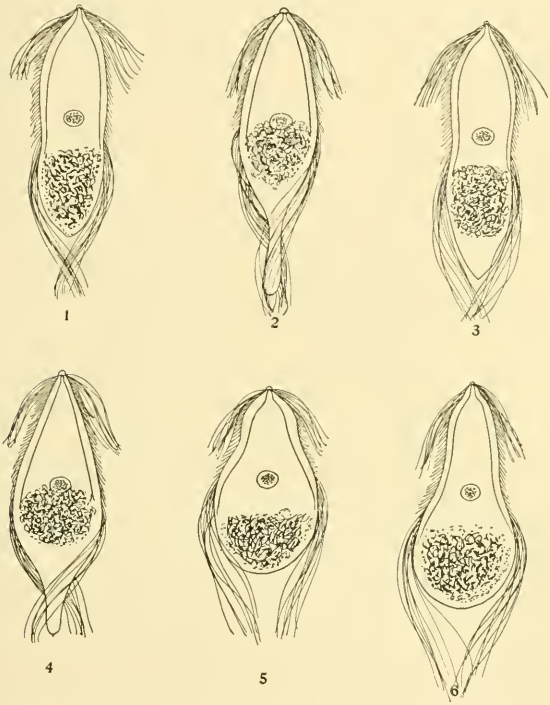

Fig. A. *Trichonympha campanula*, drawn from life, showing metabolic changes in the posterior portion of the body of the normal trophozoite. × 186.

has a transdiameter of about one-half or less than that of the posterior part. It is contracted to a slender acuminate point at the anterior end which is surmounted by a rounded, transparent, caplike structure (fig. B, *opcr.*) which we call the operculum. This covers a shallow depression at the base of which is a small pit surrounded by a darkly staining ring (fig. C, 5, 9). The base of this pitlike depression may

be protruded a short distance (fig. C, 1, 2, 8) or may be deeply withdrawn (fig. C, 5). The membrane forming the caplike covering is thin, remarkably transparent, and does not stain with any of the reagents used in the preparations of the material.

The body is radially symmetrical with graceful outlines. All trace of the characteristic asymmetry of the flagellates is lost. Its length is two or three times its greatest transdiameter which is in the posterior third of the body. The shape of the body may vary considerably from the campanulate form. The early prophases of division are marked by a characteristic rounding up of the entire body (pl. 7, fig. 31). The variation in shape in the ordinary trophozoite stage are less marked. Some of these are shown in figure A. The anterior half of the body is more stable in its outlines than is the posterior half and shows fewer metabolic changes. It may become thickened, losing the graceful curved lines, becoming conical in shape (fig. A, 4), or with a transdiameter equal to that of the posterior part (fig. A, 1).

It is in the posterior half of the body that the most striking variations are found. This is covered only with a thin periplast in contrast with the thick ectoplasm of the anterior part (fig. B). As a result of this condition it is the most delicate part and the one which most frequently shows the effects of injury in the manipulation of the material and its preparation for microscopical examination. In addition to this individuals are sometimes found which show metabolic changes in the posterior part of the organism, which are not the result of injury but may possibly be due to some chemical changes in the surrounding medium. These changes are shown in figures A, 2–4.

The posterior part may be drawn out into a slender cylinder with a consequent shortening of its transdiameter, and may sometimes equal or even exceed the length of the remainder of the body (fig. A, 2). Individuals thus affected have been observed in living material and appear normal in other respects.

In size this is the largest known species in the genus *Trichonympha*. In general length it varies from 250 to 460μ and in width from 110 to 200μ. The average length is about 350μ. A frequency curve plotted from 121 measurements had its mode at 360μ and a slight right-hand skew. *Trichonympha agilis*, as figured by Leidy (1881) and Porter (1897), varies in length from 60 to about 100μ, and for *T. hertwigi* Hartmann (1910) gives the length as between 260 and 330μ. The latter species equals in length many individuals of *T. campanula*, but falls short of the larger specimens, as well as having

a more slender body, its width varying from 40 to 60μ. In shape *T. campanula* differs but little from *T. agilis*, the latter having a somewhat broader transdiameter anteriorly with a noticeable constriction near the middle of the body or slightly anterior to it. This constriction is entirely absent in *T. campanula*. Its divergence from *T. hertwigi* is still more pronounced, varying greatly in proportions from any of the three distinct forms which Hartmann (1910) has described under that name, being more slender anteriorly and broader posteriorly.

Neuromotor System

Included in this system is the entire set of fibers concerned in movement of all parts of the body, both in the ectoplasm and the endoplasm, the external coat of cilia, the three zones of flagella, and the centroblepharoplast from which the other fibrils radiate, take their origin, or with which they have some more or less direct connection. In view of its many elements, their diversification into various groups, and their structural coördination we apply the conception of an organ system to their complex and designate it as the neuromotor system.

This system consists of two distinct parts, lying respectively in the ectoplasm and endoplasm, and differing in their staining reactions and in their relationships to the process of mitosis. The first part is composed of the flagella, their basal granules and connections, the anastomosing sheet of oblique fibers, the centroblepharoplast and paradesmose. It appears to be more highly specialized as conductile organelles, although the flagella may be sensory and are certainly contractile. This part of the system shares in mitosis, forms the polar centrosomes, the structures radiating therefrom, and the paradesmose. These organs lie in or project from the ectoplasm. The other part consists of two antagonistic sets of fibers, the outer circular and inner longitudinal myonemes. These lie against if not in the endoplasm, and take no direct part in mitosis. Their connections with the centroblepharoplast are problematical. They are primarily contractile.

The use of the term neuromotor to designate the system is based on morphological grounds and observations on the behavior of the animal. It responds to stimuli, contracts, and moves as it might be expected to do with such a structurally coördinated mechanism. It must, however, be evident that the distinction between strictly neural

and strictly motor functions can not be sharply drawn and that the two functions are in all probability not wholly separated and carried on by distinct organs, but are rather in most, if not all parts of the system, served to some extent by the same structures. It is possible that the basal ciliary lines (*b. cil.*, fig. B) are wholly conductile rather than motor, and that the centroblepharoplast has little if any motor function. It is likewise not improbable that the longitudinal and transverse myonemes are mainly motor and that the flagella and cilia are sensory, conductile, and motor, while the enveloping undifferentiated cytoplasm in which all these structures lie, except cilia and flagella, has doubtless preserved some of its primitive neuromotor capacities. The oblique fibers are by their staining reaction allied to the neural system and function rather than to the motor. These overlapping conditions, however, do not preclude the use of the term neuromotor system to designate the complex integrated fibrillar structure of *Trichonympha*.

The discussion of this system will for convenience include also that of the surrounding cytoplasmic structures, such as the surface ridges, and the alveolar layer, although these are not strictly parts of the system.

The neuromotor system (fig. B) may be divided into two very unequal parts according to its location in the two fundamental subdivisions of the cytoplasm, the ectoplasmic and endoplasmic. The ectoplasmic portion consists of the anteriorly located centroblepharoplast (*centrobleph.*) from which spring the anterior flagella (*ant. fl.*) and from which radiate posteriorly the longitudinal basal ciliary lines (*b. cil.*) which give rise laterally to the lateral cilia (*lat. cil.*) and posteriorly to the posterior cilia (*post. cil.*). These lines are in the axes of the longitudinal ridges (*surf. rdg.*) which cover the surface above the equator of the posterior region. From the centroblepharoplast arise also the spirally directed, opposing sets of oblique fibers (*obl. f.*).

One set of fibers, the circular transverse myonemes (*tr. my.*), lies in the innermost zone of the ectoplasm, or outermost zone of endoplasm. Their course is such that their connection, if any exists, with the centroblepharoplast can not be traced. In the peripheral layer of endoplasm the stout longitudinal myonemes run posteriorly from the centroblepharoplast to the margin of the thick ectoplasmic zone. No trace of any other part of the neuromotor apparatus can be found within the labile endoplasm. The juxtaposition of nucleus and cen-

troblepharoplast at mitosis is suggestive of some structural relation such as is represented by the nuclear rhizoplast in the Polymastigina, but no rhizoplast has as yet been found by us in *Trichonympha*.

ECTOPLASMIC STRUCTURES

A superficial examination of *T. campanula* reveals the fact that the anterior two-thirds of the body is marked off by a thick ectoplasm. This is thickest anteriorly and as its extreme posterior limit becomes thin, disappearing distally in the frail pellicle of the posterior region of the body (fig. B; pl. 5, fig. 6). Under the low powers of the microscope this appears as a nearly clear zone in the living flagellate, distinctly marked off from the granular endoplasm. Higher magnifications bring out the fact that it is divided into three distinct zones which are traversed by fine lines and that one layer or zone contains alveoli closely massed together. These are, (1) the outer projecting ridge, (2) the alveolar layer, and (3) the inner ectoplasmic layer (fig. B; pl. 12, fig. 80). In stained material the structure of the ectoplasmic region can be more clearly differentiated, and reveals a high degree of complexity. These regions will now be described, beginning with the outer zone and proceeding inward.

Surface Ridges: The outer surface of the body is raised in relatively high, narrow, longitudinal ridges (fig. B, *surf. rdg.*) which are best observed in a transverse section (pl. 5, fig. 4; pl. 12, fig. 80). The cilia or flagella which cover the surface of the body spring from the crest of each ridge. These ridges extend from the anterior end posteriorly to the equator. The spaces separating them become narrower anteriorly and the ridges fewer in number, finally converging around the base of the operculum-like structure at the extreme anterior tip of the body (pl. 5, fig. 1). Posteriorly they fade out at the point where the differentiated ectoplasm and flagella disappear, giving place to the thin periplast of that region of the body. The crests, or tops, of the ridges are narrow or knifelike. The base of each ridge is usually somewhat compressed (pl. 5, fig. 4). Farther posteriorly the base becomes broader (pl. 12, fig. 80) and the ridges disappear so that the entire surface becomes smooth. The ridges have been well illustrated by Porter (1897, p. 2, fig. 17) for *T. agilis*, cross-sections of the body being nearly identical in that species and *T. campanula*. Their course is longitudinal, not spiral, and is not changed to a spiral on contraction.

Locomotor Organelles: The surface of the anterior two-thirds or more of the body is covered with cilia or flagella. Both terms are not inappropriate here, since three distinct lengths of this hairlike covering is found in this species, in three distinct zones or locations. At the anterior end of the body is a zone of long, threadlike flagella (fig. B, *ant. fl.*; pl. 5, fig. 1), that may have a length equal to one-third that of the body. These arise from the narrow portion immediately behind the opereulum, directly from the centroblepharoplast or its immediate branches. Posterior to this group and extending backward for slightly more than half the length of the body is a thick covering of short, cilia-like hairs (fig. B, *cil.*; pl. 5, fig. 1) of uniform length. These have a length of one-fourth to one-fifth or less of the anterior flagella. In apparent texture and thickness they seem to be similar to the longer flagella of both the anterior and posterior regions, differing from them only in length.

The third zone of long flagella is found between the distal limits of the short cilia and the posterior end of the eetoplasmic differentiation of the surface of the body (fig. B, *post. fl.*; pl. 5, fig. 1). These are considerably longer than the flagella of the anterior zone, often having a length equal to that of the entire body (fig. A). They extend posteriorly, trailing after the body when in motion, and often intersecting (fig. A, 1) or twisting around in a loose spiral (figs. A, 2, 4).

The anterior group of flagella seems to be the chief means of locomotion. When the organism is at rest constant vibratory waves pass through the entire coating of cilia and flagella, beginning at the anterior end and passing posteriorly to the tip of the longest flagella, but dying out in intensity distally. These vibrations continue in the cilia and posterior flagella when the flagellate is in motion but the rate of movement of the anterior flagella is greatly accelerated. They are thrown out in longer and stronger vibrations, resulting in a rapid movement forward of the body.

Flagella intermediate in length between these three groups are not infrequent (pl. 5, fig. 3) near the margin of the areas. In *T. agilis*, as figured by both Leidy (1881) and Porter (1897), the entire area of short cilia is replaced by flagella intermediate in length between the short anterior group and the longer posterior flagella. This results in a thick coat of long flagella for nearly the entire body in that species.

Each flagellum or cilium in *T. campanula* arises from a minute basal granule below the ridges of the surface of the body (pl. 12,

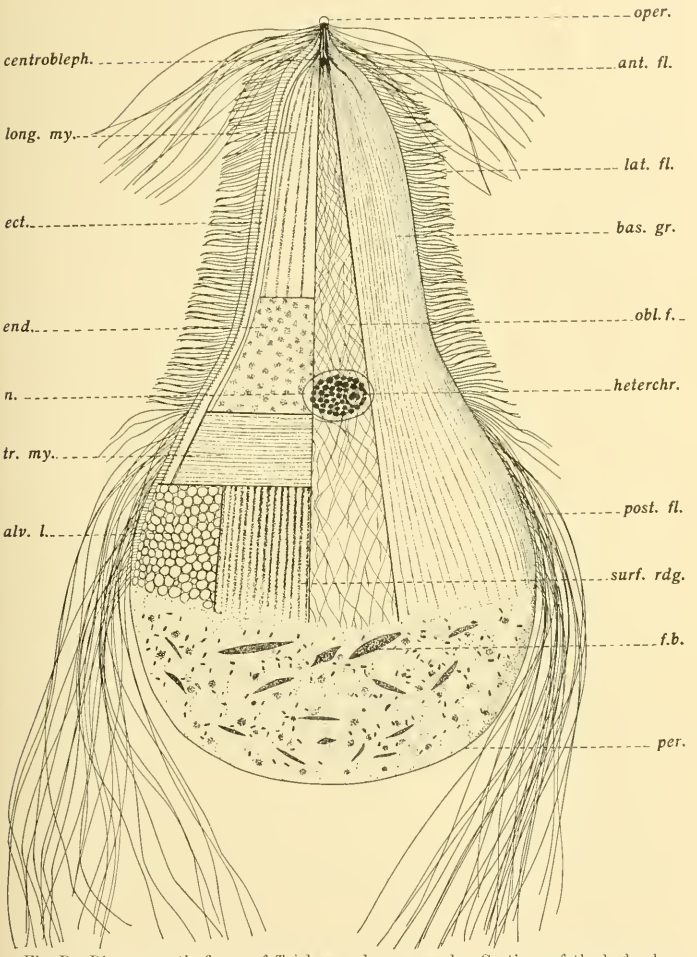

Fig. B. Diagrammatic figure of *Trichonympha campanula*. Sections of the body show the structures found at different levels. Surface ridges form the outer layer with their rows of flagella; beneath are successively the oblique fibers, alveolar layer and transverse myonemes. In the endoplasm are the longitudinal myonemes.

Abbreviations: *alv. l.*, alveolar layer; *ant. fl.*, anterior zone of flagella; *bas. gr.*, basal granules; *centrobleph.*, centroblepharoplast; *ect.*, ectoplasm; *end.*, endoplasm; *f. b.*, food bodies; *heterchr.*, heterochromosome; *lat. fl.*, lateral zone of flagella; *long. my.*, longitudinal myonemes; *n.*, nucleus; *obl. f.*, oblique fibers; *oper.*, operulum; *per.*, periplast; *post. fl.*, posterior zone of flagella; *surf. rdg.*, surface ridges; *tr. my.*, transverse myonemes. × 600.

fig. 80). Extending out from each granule is a slender thread or rhizoplast, the basal part of the flagellum. This passes up through the ridge and leaves the crest as the single flagellum. The flagella are placed closely together so that the rows of basal granules form continuous lines extending from the anterior end of the organism posteriorly (fig. B, *b. gr.*). No connection could be found between the separate granules in the same rows or in successive rows, except that afforded by the oblique fibers which will be described below. No differences could be detected between the basal portions of the three different groups of flagella.

Oblique Fibers: The most superficial examination of this species of *Trichonympha* reveals a wonderful development of myoneme-like fibers which cross and intercross in an intricate pattern over the entire two-thirds or more of the surface of the body. A closer examination brings to light three different sets of these fibers lying at different levels, and all more or less visible in the living organism. The outermost layer of these is composed of oblique myoneme-like fibers and will be described first.

These lie immediately below the surface ridges and cover the same portions of the body as do the other ectoplasmic structures (fig. B, *obl. f.*). On plate 12, figures 79, 84, and 85, these fibers are shown in dividing forms with the other ectoplasmic structures omitted. The number of fibers or separate strands is somewhat reduced in the drawing to obtain clearness, both in these figures and in others which appear elsewhere on the plates.

The oblique fibers arise from the darkly staining masses, the centroblepharoplast (fig. B, *centrobleph.*; pl. 6, figs. 7, 10), at the base of the short, narrowed anterior part of the body. Figure 7, plate 6, gives a vertical view of this region in an individual which had become rounded up, preparatory to division. This condition results in a spreading apart of the fibers, giving a clearer picture of these structures than may be obtained in the ordinary trophozoite. Thick strands stream out in all directions from the irregular borders of the centroblepharoplast, which soon break up into small threadlike branches. At first longitudinal as they leave their place of origin, this direction is lost with the first branching, the threads extending obliquely and crossing and intercrossing with one another in a complex, anastomosing network. Each intersection of two branches seems to anastomose completely so that the course of a single branch is soon lost. In addition to this network of fibers, very slender, minute

branches are being continuously given off which pass to the surface along the ridges (pl. 6, fig. 9). The basal granules of the flagella seem to be connected with these minute branches, as in optical section the flagella are seen to be continuous with the branches that are given off by the oblique fibers. In cross-sections of the body this connection could not be followed, owing apparently to decolorization in the staining methods used.

The structure of these fibers is scarcely granular and presents a more nearly homogenous appearance than is the case with either the transverse or the longitudinal myonemes. In the living organism they may be seen as very slender refractive lines. In preparations stained with iron haematoxylin they are very distinct as greyish lines, darker in the anterior region. Owing to their affinity for this stain a considerable degree of decolorization is required before they lose the black color. With Mallory's connective-tissue stain these fibers usually, though not invariably, show a clear red color similar to that found in the neuromotor apparatus of ciliates.

Centroblepharoplast: Intimately related to these fibers is another structure at the anterior end of the body which we have called the centroblepharoplast, an organelle homologous with the blepharoplast or centroblepharoplast found in other flagellates (Kofoid and Swezy, 1915), and suggestively like the motorium in ciliates (Sharp, 1914, Yocom, 1918), although that organ has no proven relations to mitosis as has this structure. Owing to the staining reactions and apparent structure of the oblique fibers first described, it seems probable that they are, of all the neuromotor apparatus, most intimately associated with the centroblepharoplast as well as with the flagella.

The anterior end of the body becomes narrow, sometimes with a slightly constricted appearance (pl. 6, fig. 6), but usually subconical in outline (pl. 6, figs. 1, 2). The ectoplasmic zone is here much thicker than in other regions of the body. In the center of the terminal cone is a slender cone-shaped structure composed of several strands of darkly staining material surrounding a central core which does not stain (fig. B, *core*; pl. 6, figs. 1, 6). This reaches the tip of the cone, where it may terminate in two ways. The first of these presents a ringlike appearance in vertical view, with the central core of endoplasm showing in the center as a light area (pl. 6, figs. 3, 7). This is a circular band around the core, to which the radiating strands of the oblique fibers are attached at their anterior ends. The second method is generally found in division stages and shows the

sides with their darkly staining strands drawn out beyond the central core and terminating in a point (pl. 6, fig. 10) with a complete obliteration of the core. Some curious modifications of this are sometimes seen in which a considerable amount of the darkly staining material has accumulated at the tip and is thrown out sideways into hornlike processes (pl. 6, fig. 9; pl. 8, fig. 34; fig. C, 6).

This axial structure extends backward for a short distance from the apex of the cone, tubular in shape or slightly larger posteriorly (pl. 5, figs. 1, 6). Near its base the separate strands, which are usually quite distinct, become enlarged into broad, irregular, darkly staining masses (fig. C) which appear to fray out around their distal margins. These may sometimes be separated (pl. 6, figs. 9, 13) into ropelike and brushlike masses, or an almost continuous band may be formed around the base of the tubular part of the neuromotor apparatus (pl. 6, fig. 7). Distally these masses break up into the oblique fibers which have been described above. The two structures seem to be continuous and composed of the same material. They connect the centroblepharoplast with the external motor organs, the flagella, by their intimate association with their basal granules.

Alveolar Layer: Closely filling the same regions traversed by the oblique fibers is a layer of alveoli (fig. B, *alv.*; pl. 7, fig. 24). This is continuous in extent with the other ectoplasmic structures, but along the posterior border it may sometimes seem to merge into the larger endoplasmic alveoli which often fill the posterior portion of the body (pl. 5, fig. 6).

In cross-section the alveoli seem fairly regular (pl. 5, fig. 4), and are placed closely together. In surface view they appear less regular with larger alveoli in the posterior region. In the rounded dividing forms this layer is often very striking, the alevoli having the appearance of clear globules much larger than those usually present in the normal vegetative forms (pl. 7, fig. 24; pl. 12, fig. 76), possibly as a result of pressure. The intra-alveolar spaces are traversed by branches from the oblique fibers (pl. 6, fig. 9) and by the basal fibrils of the flagella, the basal granules lying in the zone immediately beneath the alveoli, which is also occupied by the major portion of the oblique fibers (pl. 5, fig. 4). The zone lying between the alveolar layer and the surface is also traversed by these basal fibrils which give to that region a striated appearance (pl. 5, fig. 5). Optical sections from the posterior border of the ectoplasm in a dividing trichonymph (pl. 12, fig. 80) show the basal granules lying close

to the inner border of the alveoli. The outer zone between the alveoli and the surface ridges has here almost disappeared, the alveoli abutting on the bases of the ridges, from which they are rather widely separated anteriorly (pl. 5, fig. 4).

A distinct alveolar zone lying in the ectoplasm is a ciliate rather than a flagellate characteristic. It is not, however, entirely unknown

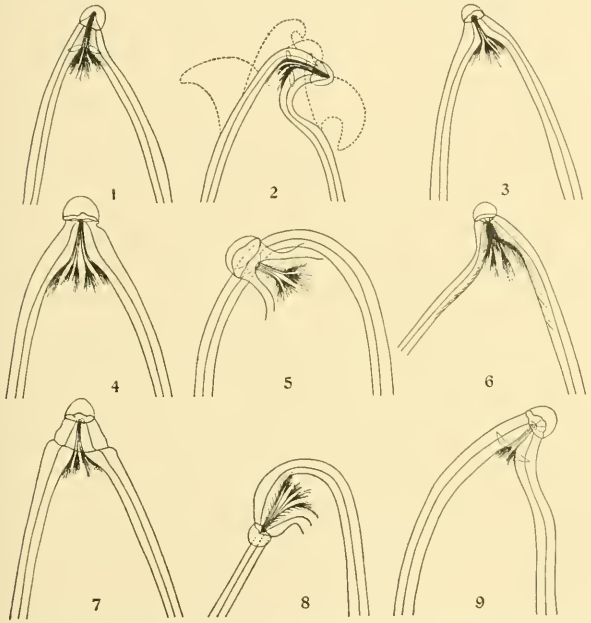

Fig. C. *Trichonympha campanula.* Sketches of the anterior end of the body to show its great mobility. Figure 2 illustrates the successive movements used in moving forward through the thick intestinal contents. × 200.

in the latter group. In the subgenus *Pachydinium* of the genus *Gymnodinium,* among the dinoflagellates, the different species are provided with a differentiated ectoplasm the outer layer of which consists of rather large alveoli (Kofoid and Swezy, 1919*d*).

Transverse Myonemes: The innermost layer of ectoplasm, beneath the alveolar and oblique fiber zone, is occupied by the transverse myonemes (fig. B, *tr. my.*). These differ in structure from the oblique

fibers, being granular in appearance as are the longitudinal ones. They are circular, passing around the body in nearly transverse planes, and may be found throughout the entire region of thickened ectoplasm. Each myoneme is a slender, granular band, not more than one or two microns in thickness. These lie in parallel rows which give a finely striate appearance to the innermost borders of the ectoplasm.

These transverse myonemes are only faintly outlined in the stained specimens and are usually obscured by the wealth of oblique fibers which overlie them.

ENDOPLASMIC STRUCTURES

In the living organism the body is marked off into three distinct regions, the outer clear ectoplasmic zone, the anterior endoplasmic region of rather dense homogeneous appearance, and the posterior endoplasm filled with coarse alveoli and food particles. The two endoplasmic regions are always more or less clearly differentiated, but without the granular layer which separates these two portions of the body in *Trichonympha agilis* (Porter, 1897). The transition from one region to the other is distinguished by a change of structure rather than by a delimiting layer of differentiated ectoplasm.

The anterior region extends from the anterior end backward for about two-thirds or slightly more of the total length of the body (pl. 5, fig. 6). Proximally a slender plug or core of endoplasm extends out through the narrow neck formed by the centroblepharoplast to the periphery of the body. This region of endoplasm is coarsely granular without distinct alveoli. The distal portion, extending up to and around the nucleus, is usually more dense, often taking a darker stain with iron haematoxylin, showing greater metabolic activity than in the anterior portion. It is also frequently filled with minute flecks of a dark color (pl. 5, fig. 6; pl. 7, fig. 23). These are often present in the posterior region of endoplasm and are possibly remnants of bacteria which have been ingested with other food particles. The possibility of their being chromidia is not excluded, however.

The posterior portion of endoplasm is filled with large alveolar spaces with the interstices occupied by coarsely granular plasma (pl. 5, fig. 6; pl. 7, fig. 23). This part of the body usually contains an abundance of food particles. As most of the termites examined were obtained from decayed wood, this was the only foreign material found in the intestine. It also seems to form at least a part of the

food of the trichonymphs, since few specimens were noted which did not contain small particles of wood in the endoplasm. The entire posterior portion of the body is often found densely filled with this material which is light in color in the unstained preparations. The particles seem to be confined exclusively to the posterior region of the endoplasm.

Longitudinal Myonemes: The longitudinal myonemes are found in the outer layer of endoplasm (fig. B, *long. my.*), a short distance below the inner layer of ectoplasm containing the transverse myonemes. These extend from the base of the tubular portion of the neuromotor apparatus posteriorly to near the end of the differentiated ectoplasmic region (fig. B, *long. my.*). They are subparallel for most of their length, spreading apart posteriorly and converging anteriorly until they meet in the region of the lobes of the centroblepharoplast. . The connection between these myonemes and this portion of the neuromotor system is one difficult to determine. The myonemes stain only faintly or not at all in the ordinary smear preparations. In sections they may be seen as dark granular masses near the outer border of endoplasm. It is in the living flagellates that they are best observed. Here they may be seen as slender bands of homogeneous appearance, somewhat refractive, and extending in an anteroposterior direction and vibrating with the movements of the body. In stained material they appear as strands of rather coarse granules which are somewhat denser and slightly darker than the surrounding endoplasm. Their structure seems to be entirely changed by the processes of fixing and staining.

The longitudinal myonemes seem to be the chief organelles concerned in flexions of the anterior end of the body. This is extremely mobile, turning easily from side to side (fig. C, 2), sometimes reaching backward upon itself until it touches the posterior portion of the body. With such movements in the living organism somewhat slowed down, these strands may be seen to sway slightly with each movement.

Nucleus: The nucleus is a rotund ellipsoid lying in the middle third of the body. Its position varies from one-third of the total length of the body from the posterior end (pl. 7, fig. 23) to about the mid-region (pl. 5, fig. 6). In a cross-section of the body at its level it lies near the center of the plane (pl. 5, fig. 4). A thin, distinct nuclear membrane separates it from the surrounding plasma. In its internal structure it presents some unusual features which differentiate it from other flagellate nuclei.

In the "resting" nucleus four parts may be distinguished (pl. 6, fig. 11). The center is occupied by a linin reticulum closely filled by a mass of chromatin granules. These are sometimes large and closely massed together, or they may be small and show a definite linear formation (pl. 6, fig. 14). In the predivision stages the latter arrangement becomes the common one and results in the formation of the chromosomes (pl. 6, figs. 14–18). Near the outer border of this central mass and often slightly imbedded within it, is a small vesicle surrounded by a very thin membrane. This contains a single small, coiled or twisted rod of chromatin (fig. B, *heterochr.*; pl. 6, figs. 11, 14, 16), surrounded by a clear area which does not stain. The position of this vesicle varies somewhat in different individuals. It is most frequently found near the end of the longer axis (pl. 6, figs. 11, 14, 19), but may lie at the side of the nucleus near the end of its shorter axis (pl. 6, fig. 16). Its size also varies somewhat as well as that of the chromatin rod contained within it which we have designated heterochromosome for convenience. In plate 6, figure 11, the vesicle is relatively small and nearly filled by the chromatin rod. In figure 16 of the same plate, both the vesicle and the chromatin rod are relatively large.

Outside of these two nuclear regions is a zone of large, clear alveoli (pl. 6, fig. 11). The walls of the alveoli seem, in some cases, to be continuous with the linin reticulum of the central area, but otherwise the two regions are distinct. The alveoli are rounded outwardly and pressed close together on their inner faces. They are filled with a clear fluid which does not stain. Outside of this alveolar zone and separating it from the outer membrane is a granular area, the rather coarse granules of which stain lightly with iron haematoxylin. This granular portion may have a width equal to half that of the alveolar zone (pl. 6, fig. 11), or it may narrow down to a thin line (pl. 6, fig. 14). In many individuals both the granular and alveolar regions may be almost indistinguishable, the central chromatin mass nearly filling the entire nuclear spaces.

In its structure the nucleus of this flagellate recalls that of *Gyrodinium corallinum* (Kofoid and Swezy, 1919*d*, pl. 10, fig. 117); the latter, however, has been observed only in the living condition. In this it presents a similar alveolar zone surrounding a central mass of granules. The presence of a small vesicle with its chromatin rod, or heterochromosome, has not been observed in this species.

GENERAL DISCUSSION

The entire absence of a cytostome in this flagellate has proven a source of some difficulty in explaining its methods of food taking. It is distinctly holozoic in its mode of nutrition, as the abundance of food particles found in the endoplasm testify. These often fill the posterior region and consist of particles of wood and bacteria, and even encysted forms of *Trichomitus termitidis*. The anterior region of endoplasm has, in all individuals observed, been entirely free from food bodies or vacuoles, with the exception of small, darkly staining rodlets which may be bacteria or possibly chromidia. The particles of wood found in the posterior region of endoplasm are often relatively huge and may be contained in a distinct food vacuole, but are usually found lying free in the plasma, without evident vacuoles.

The method of ingestion of these particles is a complete mystery. Leidy (1881), in his account of these flagellates, called attention to the presence of food bodies and the lack of any visible channel for their entrance into the body. Kent (1884), in his studies on the forms from the Tasmanian ants, decided that there was an oral aperture at one side of the body a short distance from the apical extremity. From this he traced a narrow oesophageal tract which opened into the digestive cavity at the posterior region of the body. He further states that in a medium of thinly diluted milk both the pharynx and digestive tract were frequently found filled with the milk corpuscles.

Porter (1897) attempted to confirm these observations of Kent's, both in the living animals and by means of sections of the body, but was unable to find any trace of an oral aperture. He does, however, offer another solution to this problem, that is, that the food particles are drawn to the posterior part of the body by the cilia and there ingested through the thin pellicle. Unfortunately the evidences for this are unconvincing.

Other investigators working on these forms, e.g. Hartmann and Grassi, have been equally unsuccessful in solving this mystery, nor has our own work afforded any light upon the subject. It is manifestly impossible to consider that food may be taken in at any point of the surface covered by the highly differentiated ectoplasm possessed by this flagellate. That the food is taken in at the extreme posterior portion of the body seems to be in direct contradiction to all known methods of feeding among Protozoa or elsewhere. There remains, then, the anterior end of the body to consider.

The extreme anterior tip of the cone-shaped end or head of the body reveals, in surface view (pl. 6, figs. 7, 12), a central core of endoplasm surrounded by a dark ring at the base of a pitlike depression. This central core of endoplasm extends backward through the tubular part of this darkly staining structure, the centroblepharoplast complex (pl. 5, fig. 6), and connects with the endoplasm of the body. As shown in figure 3, plate 5, this structure presents the requisites for functioning as a cytopharynx leading into the body. Its size as compared with that of the ingested food particles, would not militate against such a supposition, since the great flexibility of these parts might also be correlated with a considerable degree of elasticity permitting distension. That the centrosome should form part of the mouth structures, however, seems hardly plausible, but scarcely less so than that its food should be taken in at the posterior end of the body. The fact that the operculum appears always to be intact and to cover over the anterior tip of the body militates against this interpretation.

An analogous condition, in case the core is the gullet of *Trichonympha*, is found in *Diplodinium* (Sharp, 1914), where a ring of neuromotor material and connections surrounds the gullet. There is no evidence, however, that this ring has the remotest relation to any centrosome of this ciliate. Ciliates are, moreover, not mononucleate as is *Trichonympha*.

Porter (1897) has described for *Trichonympha agilis* a peculiarity in the structure of the anterior part of the body, which might afford some basis for the view of Kent that a cytostome existed in this region. He described the cone-shaped end or "nipple," as he terms it, as separated from the remainder of the body by a deep constriction, the central axial rod forming the only means of union between the two parts of the body. This appearance is shown in our own material also (pl. 5, fig. 5) but Porter's explanation of the structure of the body at this point does not agree with the actual conditions as we find them. Our own interpretation follows.

At the base of the tubular portion of the centroblepharoplast and abutting upon its lobes, is a clear area that forms a ring completely surrounding the tube. This is not traversed by the fibers that give to the remainder of the ectoplasm a striate appearance, nor is it granular in its composition. In some individuals this lack of myonemes and fibrils may be seen to extend to the outer surface which then shows a zone devoid of flagella covering this region. Usually the outer zones

are completely filled by the wealth of fibrils which crowd this part of the ectoplasm. In no case, however, have the lines bordering the different layers of the ectoplasm, which here is unusually thick, been found to be broken, or otherwise to present any indications of a constriction in the surface of the body at this place. Were this the case, flexions of the head or cone, such as are common in many individuals on every slide, would betray the lack of continuity on one side at least, and no instance of this has been observed, though the flagellates have been thrown into every conceivable attitude in making smear preparations. The surface lines or ridges on the cone also seem to be continuous with those of the body without a break in their continuity. We find no evidence of an anterolateral cytostome.

What purpose this circular vacuole may subserve, finds no explanation in observations we have been able to make. It is almost or quite obliterated in many individuals and always disappears at the time of division. Its conspicuousness when present in stained material, with its complete lack of structural differentiation in the midst of a highly differentiated zone, would suggest that it is a fluid-filled vacuole of non-stainable substance but gives no further aid in explaining it. That it might function as a cytostome seems impossible.

Evidences of the complex ectoplasmic and neuromotor structures which we have described above, are to be found in the figures of Porter (1897) for *Trichonympha agilis*, and in those of Hartmann (1910) for two, at least, of the species he assigns to *T. hertwigi*. In the former the alveolar zone of ectoplasm with the surface ridges, and something of the fibrillar system with its centroblepharoplast, are shown. In two species which Hartmann has figured, the centroblepharoplast and suggestions of the complex of myonemes may be found. The complete minute structure has in no case been worked out heretofore, neither has the presence of an integrated system been noted nor its relation to mitosis demonstrated.

BINARY FISSION

Binary fission and mitosis in *Trichonympha campanula* present some interesting phases, both in regard to cytoplasmic structures and mitotic phenomena, the latter bearing some striking resemblances to certain stages in the mitosis of the metazoan germ cells. These processes will be discussed separately, beginning with the division of the centroblepharoplast and related ectoplasmic structures.

Division of the Neuromotor Apparatus and Ectoplasmic Structures

The onset of division is marked by certain nuclear changes that will be discussed later. The first evidences of it in the grosser structures of the organism are found in the change from a bell-shape to a spherical contour in the body as a whole (pl. 7, figs. 24, 31). With this change the nucleus migrates anteriorly until it comes to lie immediately below the centroblepharoplast (fig. 31). No trace of a connecting rhizoplast has been found. This change in the form of the body results in a spreading apart of the myonemes and fibrils of the ectoplasmic layers, with an apparent enlargement of the alveoli, so that these structures are more easily studied in this stage than in the more usual but contracted form of the normal vegetative trophozoite.

The next step in the division process is found in the partition into two parts of the lobes at the base of the tubular portion of the neuromotor system, the centroblepharoplast. With this occurs a separation of the entire ectoplasmic layer into two parts, with a small, spindle-shaped portion of endoplasm appearing in the chasm thus made (pl. 7, fig. 30; pl. 8, fig. 35). At about the same time or slightly earlier, the operculum-like, transparent cap with the cup-shaped depression which it covered, disappears, and the tip of the tubular neuromotor apparatus reaches quite to or near the outer surface of the anterior end of the body.

The splitting or division of the tubular part of the neuromotor apparatus or centroblepharoplast proceeds from the base anteriorly to the tip (pl. 8, figs. 33, 34), and with the splitting each half draws together its parted edges until they meet and each moiety forms a new tube. The attachment to each other at the tips may persist for some time, as such stages are more common than are the intermediate stages shown in plate 8, figure 33. As the two halves of the tube separate, strands of darkly staining material are found joining the two inner surfaces near their bases (pl. 8, fig. 34). This is the paradesmose which functions in the formation of the spindle, and is apparently drawn out from the material of the centroblepharoplast itself. With the final separation of the tips of the new daughter tubes the paradesmose remains as the only connecting link between the two structures (pl. 8, fig. 36).

As the two parts of the centroblepharoplast separate, the alveoli frequently form a rosette at the base of each (pl. 8, fig. 38; pl. 9, fig. 42), presenting, especially in a focus showing the oblique fibers, striking resemblances to well developed asters in optical section. With the further separation, the angle formed by the splitting ectoplasmic structures becomes greater and extends farther out towards the periphery of the ectoplasmic region (pl. 8, fig. 38). This region is usually conspicuous since the flagella as well as surface ridges, myonemes and alveoli have been drawn aside, leaving only the granular endoplasm beneath the thin ectoplasmic layer. The paradesmose becomes thicker and broader, generally forming a heavy band that persists throughout the entire process of division, and may occasionally be found for some time after the final separation of the daughter nuclei (pl. 11, fig. 75).

No other changes in the ectoplasmic structures during these stages have been detected. The staining reactions of the neuromotor apparatus seem to vary slightly as the different phases follow each other. At the beginning of its division it often presents a greater affinity for iron haematoxylin than during the later phases. This, however, may possibly be due to changes in the preparation of the material, as decolorization of slides containing mitotic figures was carried on with the view of obtaining the best possible results for the chromosomes, without regard to the remainder of the cell.

In the final stages of division, when the complete separation of the divided organelles has taken place, an enveloping movement of the ectoplasmic zone may be seen. This begins with a gradual creeping out of the margins of the divided area (pl. 12, fig. 76), partly as a result of the spreading of structures already formed and partly as new outgrowths over the undifferentiated intermediate zones. As the two daughter organisms separate, pulling out a long protoplasmic bridge between them, the rounding-up of the body which results aids in closing the gap between the margins of ectoplasm (pl. 12, fig. 79). This process may, however, be hastened or retarded in some individuals, and is not always synchronous in the two daughter organisms. This possibly results from contractions of the intermediate zones or of the old differentiated zones. In plate 12, figure 85, an almost complete union of the borders of the ectoplasm has taken place in one individual of the dividing body, while the other still shows a rather broad gap between the margins of ectoplasm. In figure 84 of the same plate the final separation of the two daughter organisms has

taken place with only a comparatively small amount of new ectoplasm formed in one individual.

The behavior of the neuromotor apparatus during mitosis is significant in its intimate association with the nucleus. The entire extra-nuclear mechanism which consists of polar centrosome, the astral rays attached thereto and the paradesmose which is stretched out between them as an extranuclear band of large size, is all a direct transformation of the most intimately connected parts of the neuromotor apparatus, the centroblepharoplast, the oblique anastomosing fibrils, the lines of basal granules and the attached flagella and cilia. The longitudinal and transverse myonemes simply part at the zone of bipartition of the ectoplasmic territory, without forming an integral part, with structural modifications, of the nuclear figure of mitosis. The comprehensive fashion in which the sum total of the neuromotor system, excluding myonemes, forms the extra-nuclear mechanism of mitosis, is instructive in the matter of the unity of the system, and its integration into an organic complex which survives the shock and readjustments contingent upon mitosis without dedifferentiation and reorganization. This is in marked contrast with the extent of such dedifferentiation and reorganization in the multinucleate Ciliata, such as *Euplotes* (Yocom, 1918).

Another feature of cytological significance is the derivation of the astral rays of the nuclear spindle from what are structurally distinctly fibrillar organs, the anastomosing oblique fibers. The ciliary lines which are more granular in appearance in stained material, more homogeneous and distinctly fibrillar in life, also form radiating lines in semicircle from the poles of the paradesmose. The resemblance to the aster of dividing metazoan cells is so striking that one is inclined to regard them as homologous structures. If so, Chambers' conclusions (1917) that the asters are sol phases of the surrounding cytoplasmic gel present considerable difficulties, as does likewise the fact that these fibers in *Trichonympha* lie in one superficial plane in the surface of the organism, while the asters of the Metazoa are infiltrated through the mass of the cytoplasm in three dimensions to a much wider extent. The latter difficulty is not insurmountable since it is a logical consequence of the structural specialization of the cell which is the whole undivided trichonymph. It may also be true that with so universal a phenomenon as mitosis pervading all types of living substance, we should expect to find the bipolar organization which appears in the nuclear figure utilizing not one but many diverse

structural units in its make-up, according to the nature of the cell in which it occurs—even so diverse as the neuromotor system of a flagellate and the sol phase of reversible cytoplasm. It is the organization rather than the state of the substance that is significant.

Mitosis

Evidences of the approach of division may be looked for in the nucleus before any changes may be apparent in either the ectoplasmic structures or the external form of the body. The changes that take place in the nucleus relate to the formation of the chromosomes, and are significant both from the standpoint of their later history and of the question of the continuity of the chromosomes. Their development will now be taken up in detail.

Prophase: The structure of the vegetative nucleus shown in figures 11 and 14 of plate 6, has already been described. The central masses of chromatin seem to lie at the intersections of the linin reticulum, in some cases (fig. 11) the individual masses being large enough to completely fill the interstices also. In the early prophase the outer alveoli disappear, leaving a clear space with a granular region near the membrane (pl. 6, fig. 15). This granular material later becomes diffused through the entire intranuclear spaces and persists throughout mitosis. The chromatin moves out from the rounded particles along the lines of the reticulum, sometimes before the alveolar zone has disappeared (fig. 14). This movement becomes more evident with the change in the outer region of the nucleus, and a slight expansion takes place, with the chromatin filling a larger area than is usual in the vegetative stage.

The outpushing of chromatin from the central masses is not equal in all directions. This may be due to a lack of continuity in the linin reticulum or to its breaking up. The latter seems the more probable explanation, as threadlike ends are frequently seen in these stages (fig. 17). This breaking-up appearance begins at one side of the nucleus while the remainder still shows a close reticulum thickly encrusted with chromatin (pl. 6, fig. 15; pl. 7, fig. 26). As the breaking up proceeds further the rounded masses gradually disappear, the chromatin apparently moving out along the threads which assume a thicker, more compact appearance.

As these threads become differentiated some evidences may be found of a longitudinal split in each one (pl. 7, figs. 25, 27, 28). This may occur in some threads while the remainder are still emerging

from the undifferentiated mass of chromatin-encrusted network, hence the number of threads originally formed can not be determined. The separation of the two parts thus formed seems to take place immediately, since in slightly later stages the number of threads appears much greater, with no evidences of the splitting of a single thread (pl. 6, figs. 20–22; pl. 7, figs. 23, 29). In these stages also many of the threads or chromosomes are arranged in pairs which are nearly equal in size and length. In a later stage, but still one which precedes the rounding up of the body, this pairing of the chromosomes becomes more pronounced (pl. 6, fig. 18). The threads thus sorted out in pairs are evidently the products of the splitting of the original threads or chromosomes.

During the time these changes in the central nuclear mass are taking place, the small vesicle with its single coiled, chromatin thread or chromosome remains intact, with no apparent change beyond an enlargement of the vesicle itself. This forms a large clear area with the chromosome contained within it loosely coiled or V-shaped (pl. 7, fig. 29; pl. 6, figs. 16, 20). In common with the remainder of the chromatin of the nucleus, it seems to increase somewhat in bulk during the early prophase, though this is not invariably the case (pl. 9, fig. 46). The vesicle disappears before the end of the prophase, leaving the single chromosome lying in a clear space apart and detached from the remainder of the chromatin threads (pl. 6, fig. 22; pl. 7, fig. 29). This isolation is apparently retained throughout the subsequent stages, though this can be satisfactorily demonstrated only when the nucleus is oriented so that it is seen near the lateral margin (pl. 9, figs. 46, 47). In other positions its relations are obscured by the great number of chromosomes.

The occurrence of a definite, continuous spireme stage is not certain, the short chromatin threads or chromosomes apparently being formed directly from the breaking up of the reticulum (pl. 6, figs. 14–22), before the body of the flagellate has begun to round up or give other evidences of the approach of division (pl. 7, fig. 23). The exact number of the threads thus formed seems to be fifty-two. In the stages represented in figures 18 to 22, plate 6, these could not be counted, but in the later stages, represented by figures 44, 47 and 49, plate 9, with the chromosomes more fully organized, this could be done with a considerable degree of accuracy, and the number of chromosomes given is based on counts made on fifteen different individuals.

Each chromosome consists of a long thread composed of chromomeres closely strung together (pl. 6, figs. 20–22; pl. 9, fig. 44). In earlier stages these appear diffuse but later become more compact, at the same time drawing together at the ends to form a loop (pl. 9, figs. 46–49). These loops may appear coiled together (fig. 46) or may preserve a V-shape. Both of these appearances may be seen in the same nucleus (fig. 49).

Soon after the appearance of definite chromosomes the flagellate begins to round up, with an anterior migration of the nucleus (pl. 7, fig. 31). This is followed by the splitting of the centroblepharoplast and the separation of the two halves which remain connected by the darkly staining paradesmose (pl. 8, fig. 33).

The nucleus at this stage is found a short distance below the base of the dividing neuromotor system (pl. 7, fig. 30). The chronological relation of the changes occurring in the nucleus and those of the neuromotor apparatus vary considerably. The stages shown in figures 20 to 22, plate 6, usually occur before the centroblepharoplast divides, yet occasionally the paradesmose may be fully formed before definite chromosomes appear. The formation of the spindle fibers immediately below the paradesmose does not occur until the nucleus approaches the paradesmose with the nuclear membrane apparently touching it. The spindle fibers are stretched between the dark masses or centroblepharoplasts at either end of the paradesmose (pl. 9, figs. 41, 43), but inside the nuclear membrane. When these are fully formed the nuclear membrane is drawn out to a spindle shape with the ends reaching the ends of the paradesmose (pl. 10, figs. 55–59). The latter structure remains outside of the membrane but closely pressed against it, usually partly imbedded within a fold, which in many views gives it the appearance of occupying the center of the spindle and chromosomes (pl. 10, fig. 55). In reality, however, the chromosomes and spindle fibers are at all times completely separated from it by the nuclear membrane. Its position thus approaches that of the centrodesmose or central spindle of the metazoan cell. Since it is outside of the nuclear membrane it is a paradesmose.

With the beginning of the formation of spindle fibers or somewhat earlier, another change takes place in the chromosomes, the loops straightening out so that the chromosomes come to lie parallel to the paradesmose. This process may be followed in figures 56 to 59, plate 10, with the chromosomes in various stages of unbending. The completion of this gives the equatorial plate phase (pl. 10, fig. 59),

with the chromosomes still joined by an end to end union in the equatorial plane. The behavior of the heterochromosome is not always easy to determine in this stage. In figure 56, plate 10, it is found lying near one end of the mass of chromosomes. Its attachment to distinct spindle fibers could not be demonstrated. In figure 59, plate 10, it has moved nearer the equatorial plane but is still outside the main mass of chromosomes.

Metaphase: The equatorial plate is wide and usually heavily stained, with the chromosomes closely massed together. It is apparently of shorter duration than either the prophase or later stages. With the elongation of the nucleus the chromosomes separate at the middle point, i.e., at the apex of the looped thread or V of the chromosome before it became attached to the spindle (pl. 10, fig. 60; pl. 11, fig. 62).

During the later prophase the small chromatin thread or heterochromosome is often obscured, particularly in the formation of the equatorial plate. With the separation of the chromosomes in the metaphase, however, this again becomes prominent. It is found that the vesicle has disappeared and the single thread has divided (pl. 10, fig. 61). The separation of the two new heterochromosomes in the metaphase lags somewhat behind that of the other chromosomes, hence these usually may be seen between the two groups as they pass towards the poles (pl. 11, figs. 62, 64). The attachment of these chromosomes to spindle fibers in these stages, as in the earlier ones, has not been determined.

Anaphase: The separation of the chromosomes after the final parting seems to take place by reason of an elongation of the entire nucleus in the equatorial region, rather than by a shortening of the spindle fibers, since the chromosomes have in no case been found closely attached to the poles. The spindle fibers remain approximately the same length throughout the anaphase until they disappear in the telophase (pl. 11, figs. 61–65, 73, 74). The elongation of the nucleus in the equatorial region is accompanied by a constriction of the nuclear membrane. It is usually drawn out into a long, slender strand before the final break occurs which separates the two daughter nuclei (pl. 11, figs. 71, 72). At the same time the paradesmose also lengthens as the centroblepharoplast complexes of the newly forming daughter cells move farther apart (pl. 11, figs. 68, 72, 75).

The connecting nuclear thread thus formed soon breaks and the elongated portion of each daughter nucleus is gradually withdrawn

(pl. 11, figs. 70, 73, 74), the nuclei becoming rounded or nearly so, with a separation from the paradesmose and the centroblepharoplast (fig. 75). The paradesmose loses its staining reactions and soon fades out, apparently being resorbed, either in the cytoplasm or by the centroblepharoplast complexes. The chromosomes in the meanwhile undergo few or no changes, retaining a position at some distance from the poles after the disappearance of the spindle fibers, which may not occur until the nucleus has begun to round up.

Telophase: The reorganization of the nucleus may take place before the constriction of the cytoplasmic body (pl. 12, figs. 76, 79), or it may be delayed until after separation of the two daughter cells (fig. 84). The heterochromosome may be found at this period lying near the ends of the chromosomes opposite to their point of attachment to the spindle fibers (pl. 11, figs. 73–75).

A vesicle is formed about this (pl. 12, fig. 83), and the other chromosomes become aggregated in the central part of the nucleus. These gradually lose their parallel positions and become mingled in a coarse network (figs. 83, 78, 81) which resembles in many respects a similar stage of the prophase nucleus (pl. 6, fig. 16). The nucleus may begin its migration away from the centroblepharoplast to the posterior region of the body even before final constriction of the cell (pl. 12, fig. 76), though this is usually delayed until the daughter flagellates begin to assume their elongate, campanulate form.

Discussion of Mitosis

Observations on the division of the members of this unique genus have been scanty heretofore. Foà (1904) describes and figures some stages of this process in two species which she designates as *Trichonympha agilis* forma *minore* and *T. agilis* forma *maggiore*. The stages she has figured are strikingly similar to the same stages found in our own material.

In both of these species division is preceded by a rounding up of the body and an anterior migration of the nucleus, followed by division of the anterior, narrowed portion of the body, the "tubolo," and the ectoplasmic zone of flagella. As these structures separate they spin out between them a stout band, the external spindle (*fuso esterno*). The internal spindle (*fuso interno*) is formed by fibers arising from the ends of this, passing through the nuclear membrane and attaching themselves to the chromosomes. The remaining steps

in the process, so far as Foà has described them, are identical with those of *Trichonympha campanula*. She does not, however, give further details of the process, the formation of the chromosomes or their number. She did not record division of the chromosomes in the small form but figures longitudinal splitting in those of the larger species.

In the nearly allied form, *Holomastigotes* (*Trichonympha hertwigi*) Hartmann (1910, pl. 28, figs. 23–29) has figured certain stages of the prophase nucleus, leading up to the formation of the chromosomes, which are nearly identical with phases found in our own material (pl. 6, figs. 14–16, 20–22). He also figures division of the structure which we call the centroblepharoplast and the anterior tip of the body, processes which also closely parallel those found in our own material. Further details of the mitotic phenomena he did not record.

The work of earlier investigators of these flagellates, such as Leidy (1881) and Porter (1897), also fails to give any clue to the details of the division processes.

The abundance of division stages in our own material has given us an unusual opportunity of determining the flagellate type of mitosis in *Trichonympha* and to follow out the details of the mitotic phenomena. The relatively large size of the nucleus of *Trichonympha* and the structures connected with its division, renders it a favorable object for study. In the foregoing outline of these various processes, certain points have been omitted or briefly touched upon, which will be discussed more fully in the following paragraphs, along with an explanation of some of the terms used in this paper.

The union of blepharoplast and centrosome in one structure which may or may not become separated at the time of division, is a condition quite common throughout the flagellates generally. The occasional separation of these in trichomonad flagellates (Kofoid and Swezy, 1915), becomes a permanent condition in the mitosis of *Trichomitus termitidis* (Kofoid and Swezy, 1919*b*). This relationship makes the term centroblepharoplast an appropriate one for this structure. The application of it to the more complex organelle of *Trichonympha* is also based on these same relations. The enormous increase in the number of flagella in this organism necessitates a coördinating mechanism related to each flagellum individually. This is found in the intricate system of fibrils radiating from the central mass at the anterior cone-shaped portion of the body, which thus

becomes a huge blepharoplast complex. At the time of division the entire structure divides into two parts, taking the rôle of centrosomes in the succeeding mitotic figures, while continuing its intimate relations with the flagella. The term centroblepharoplast thus designates the dual functions of this organelle complex.

Equally distinctive and typical of the phenomena of mitosis in flagellates is the formation of a paradesmose conecting the divided centroblepharoplasts. In *Trichomonas* (Kofoid and Swezy, 1915) at the occasional separation of the blepharoplasts and centrosomes this structure appears to be connected with the blepharoplasts and not with the centrosomes. In *Trichomitus* (Kofoid and Swezy, 1918*b*), however, the paradesmose is found connecting the centrosomes to which the blepharoplasts are attached by a slender rhizoplast. In this form these structures have a longer lease of life, the majority of individuals noted showing the prophase stage with the completion of the formation of the paradesmose. In both these flagellates, as in *Trichonympha*, it subserves the same function in mitosis with the same relative position *outside* the nuclear membrane.

The heavy band connecting the two parts of the divided centroblepharoplast in *Trichonympha*, to which Foà (1904) has given the name external spindle (*fuso esterno*), we consider homologous with the paradesmose of the trichomonad and other flagellates, and have so designated it. We have given a fuller discussion of this subject in an earlier paper (1919*c*).

The similarity of the paradesmose and the "sphere" of *Noctiluca* points to an homology between them, which thus links what has been considered a peculiar type of mitosis (Calkins, 1899) in the latter form with conditions found among other flagellates. Whether the nuclear membrane dissolves at the points of contact with the paradesmose in *Trichonympha*, as in *Noctiluca*, has not been definitely ascertained, but no evidences to support such a conclusion have been found. The ultimate fate of the paradesmose in both *Noctiluca* and *Trichonympha* seems to be the same, that is, it fades out in the middle and is absorbed or drawn up into the central mass of the centrosomes.

In the small chromatin rod, isolated from the remaining chromatin contents of the nucleus, we have a structure that is unique among the Protozoa and finds its nearest counterpart in the Metazoa in the "sex" chromosomes of the germ cells. Its resemblances to the latter are particularly striking during the different phases of division.

Its significance is problematical. No evidences of sex or sexual behavior have thus far been found in these flagellates. Hartmann (1910), it is true, has described both male and female forms in his *Trichonympha hertwigi*. His observations, however, do not bear out this assumption. No critical evidences of conjugation were found by him, neither in the behavior of conjugating gametes nor in the nuclear changes which precede this process. Indeed, it is evident to anyone familiar with taxonomic conditions in the Protozoa, that his male and female forms belong to different genera, as has already been pointed out by Grassi (1911). In his "junge, männliche, and weibliche" forms he has confused three distinct species and even genera, with a fourth species added to this confusion in the "gametes" which are minute oval flagellates such as are frequently abundant in the intestinal contents of many termites. His elaborate life cycle of this form is thus seen to be without adequate foundation, the product of an overwrought creative imagination.

This lack of evidence of sexual behavior in these organisms renders doubly difficult any explanation of the significance of the function of this peculiar structure. It remains distinct throughout both the vegetative and division cycles, dividing in the metaphase, its position that of a lagging chromosome in the anaphase, with one part going to each daughter nucleus. At some stages it bears a strong resemblance to the chromatoid body described by Wilson (1913) in the sperm cells of *Pentatoma* and some other insects. Its further behavior, however, clearly distinguishes it from that body, which is cytoplasmic in origin and does not divide. In *Trichonympha* this body has never been found outside of the nucleus and behaves as do other chromosomes at the time of division. As a convenient designation for this body and one which leaves its specific function still open to investigation, we have used the term heterochromosome, since that word, though originally used for the sex chromosome, has come to be applied to other forms of chromosomes as well (Wilson, 1911). The possibility is still open that further investigations may find undoubted evidences of sexual behavior in these flagellates.

Certain other aspects of mitosis in *Trichonympha* show striking resemblances to the mode of procedure found in the division of metazoan germ cells. The most remarkable of these is found in the "pairing" of the chromosomes with a reduction of their number from fifty-two to twenty-six (pl. 9, figs. 44–51*a*). This pseudosynapsis, however, may be explained on grounds other than that of sexual behavior and our interpretation of it follows.

The formation of distinct chromosomes takes place in the nucleus some time before any signs of division may be detected in the remaining structures of the body (pl. 7, fig. 23). The nuclei shown in figures 14 to 22, plate 6, and figures 25 and 26, plate 7, show different steps in this process. The number of threads or chromosomes that result has not been made out clearly at this stage. With

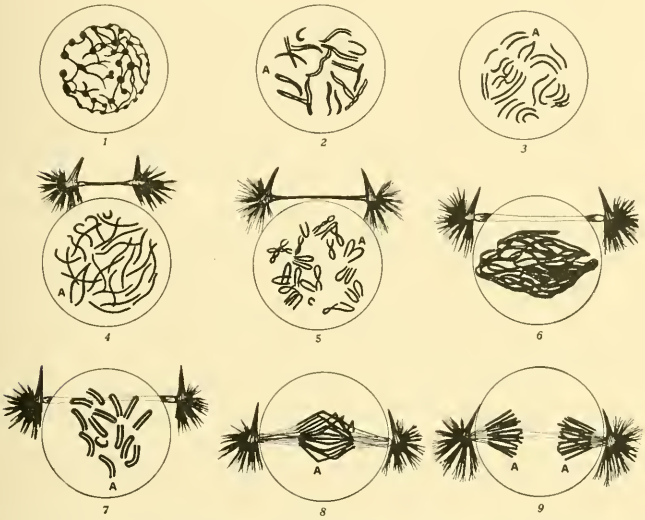

Fig. D. Diagram illustrating the phases of nuclear mitosis in *Trichonympha campanula*. One-half the number of chromosomes is shown. 1–7. Prophase. 1. Vegetative phase of chromatin-encrusted network. 2. Splitting of the chromosomes. 3. Separation of chromosomes resulting from splitting but paired arrangement noticeable. 4. Paradesmose formed between daughter centroblepharoplasts. 5. Formation of loops; nucleus approaching elongated paradesmose. 6. Tangled stage in which pseudosynapsis occurs. 7. Number of chromosomes reduced one-half. 8. Metaphase; looped chromosomes unfolding on the spindle. 9. Late anaphase; paradesmose still connecting centroblepharoplasts. Chromosome marked A is splitting in figure 2, appears as two chromosomes in 3 to 5, is reunited in 7 and 8, and separated into two distinct chromosomes in 9. The small coiled chromosome is the heterochromosome.

their *first* appearance, however, signs of splits in the threads may be detected (pl. 7, fig. 25; text fig. D). In the following stages these threads become clearer and their number may be ascertained. The nucleus is then found to contain fifty-two chromosomes arranged somewhat in pairs (pl. 9, fig. 44; text fig. D), that is, the end of one

chromosome will be found near or attached to the end of another, though the opposite ends may be widely separated. This is shown more clearly in the diagrammatic scheme in figure D, where one-half only of the actual number of chromosomes has been drawn in each nucleus. Each of these groups of two chromosomes is probably formed by the splitting of a single thread in the earlier stage (fig. D, 2).

Following this, each chromosome becomes looped or doubled upon itself (fig. D, 5). In this stage also it is found that there is some suggestion of a grouping of the chromosomes in pairs (pl. 9, figs. 46, 49), though the ends may be more widely separated than in the previous stage. Following this the chromosomes condense into a tangled mass of threads or contraction stage (fig. D, 6; pl. 9, figs. 42, 43, 45, 50), from which emerge twenty-six looped or V-shaped chromosomes (fig. D, 7; pl. 9, fig. 51*a*; pl. 10, figs. 52, 53). What happens in this contracted condition can only be conjectured, since thus far the details have escaped detection.

When the chromosomes divide in the metaphase the point of separation occurs at the apex of the V or loop. If division here is longitudinal, and the evidences of the earlier stages (fig. D) confirm this view, the line of separation must be at one end of the original split found in the chromosomes when first formed. This would postulate the supposition that the two halves of the original chromosome are reunited at the time the change in the number of units from fifty-two to twenty-six occurs. To illustrate this, let us follow the course of the chromosomes marked A in figure D. In figure 2 the chromosome A is a single thread that has partially split. This is completed in figure 4 but the ends of the chromosomes are still connected. In figure 3 this connection has apparently been lost, for the ends are separated by a considerable space. The course of these two threads is lost in the contraction stage in figure 6 but they emerge again in figure 7 as a single split chromosome which parts "transversely" on the spindle in the following figure, the two threads having apparently become united end to end into one, in the stage represented by figure 6. The seemingly transverse division is in reality the final step in longitudinal splitting of the original chromosome.

The only difficulty in this explanation lies in the lack of a visible mechanism by means of which two threads which are more or less widely separated from each other, are reunited, with their relative positions those of the original split. This difficulty is, however, not

so formidable as would seem at first glance. A single chromosome is evidently composed of a ground substance, framework, or thread of linin in which the chromomeres are imbedded and which gives such great consistency to the entire structure. This does not stain and its presence is difficult to demonstrate satisfactorily except as it is outlined by the stained chromatin.

At the time of division of the chromosomes in *Trichonympha* (fig. D, 2; pl. 7, fig. 25), a physical continuity may still be retained between the two threads thus formed, by the incomplete division of this framework. This invisible link, whose existence is suggested by the behavior of the ends of the chromosomes, would serve to keep one end of each chromosome near the corresponding end of its fellow, and would explain the apparent pairing of chromosomes in these stages (pl. 9, figs. 46–49). These stages, however, have not been observed in the living cell and it is possible that the spreading apart of such pairs may be due to the manipulations of the operator in making smears in the preparation of the material. It is conceivable, however, that considerable separation or strain may occur in the chromosomes of the normal cell without reaching the point of complete separation.

Given this physical continuity the drawing together of the two threads and their condensation into two shorter parallel threads joined at the apex, becomes a simple matter. Such precocious splitting and separation and their subsequent union before going on the spindle, have been found to occur in the chromosomes of the trichomonad flagellates (Kofoid and Swezy, 1915) and in *Giardia* (Boeck, 1917), a procedure which would seem to support an explanation similar to that given above. In view of our present knowledge of cytology, the alternative explanation would be that we have here a synapsis of chromosomes occurring in the ordinary vegetative division cycles, since it is highly improbable, even were these flagellates found to be sexually differentiated, that all the division cycles found within a single year, would be only those of gametes and not the ordinary trophozoite division, where such a reduction in the number of the chromosomes would not be expected to occur. The possibility that the behavior of pregametic chromosomes in the Metazoa is a specialization of a more widely prevalent phase of mitosis in the Protozoa is not precluded.

Evidences for the precocious splitting of the chromosomes immediately following the end of a division period, have been carefully searched for, but thus far have not been found. At what period in

the "resting" phase of the nucleus this occurs has not been determined. The abundance of such stages in what appear to be normal vegetative forms, without other signs of the approach of division, would suggest that it occurs very early in the between-division periods of the life of the organism.

That we may have here a continuity of chromosomes from one division period to the next is suggested by certain appearances of the resting phases of the nucleus. This is best seen in figures 14, 15, and 17 of plate 6, and figures 26 to 28 of plate 7. These are the nuclear figures which are most frequently met with in the ordinary trophozoite. They present a broken, ragged network with distinctly marked ends of chromatin threads scattered through it. Occupying the nodes of the network or sometimes at the ends of the threads, are chromatin granules. The process of changing from this condition to that of the distinctly marked chromosomes seems to consist in an outmoving of the contents of the granules along the threads to which they are attached (pl. 6, figs. 17, 19). On the completion of this, the fully formed chromosomes become apparent, with an entire absence of large chromatin granules. Differentiation of individual chromosomes other than the heterochromosomes has not been detected.

In the late prophase of division the chromosomes retain distinct outlines for a considerable period. With the disappearance of the spindle fibers the chromosomes move out to near the center of the nucleus, with the threads lying parallel or nearly so (pl. 12, figs. 77, 82). Without apparently losing their individuality, these separate threads begin to change their position (fig. 78), and form a loose network by the interweaving of the separate strands (figs. 78, 81, 83). Later granules appear at the intersection of the threads which then may become thinner and we have the same nuclear structure as that shown in figures 27 and 28 of plate 7, and figures 14 and 15 of plate 6. Distinct chromomeres cannot be detected in these stages of the nucleus, but they appear later following the formation of distinct chromosomes.

The type of division of the chromosomes is apparently transverse in the later stages, but is in reality longitudinal, as shown by the longitudinal splitting of the single threads (fig. D; pl. 7, fig. 25). The contraction of the two, visibly separated halves of the chromosomes (pl. 9, fig. 49) into single short, thick threads with a V-shape (fig. 51*a*), produces the units which are unfolded on the spindle as long, single threads (pl. 10, figs. 56, 59). These part in the middle,

at the apex of the original V, and thus finally complete the original splitting. Division of the chromosomes is thus in *Trichonympha*, as in other flagellates, a fundamentally longitudinal process.

In the division of the protoplasmic body the same longitudinal type of division also holds true. In the rounding up of the body the anteroposterior relations are somewhat obscured. The beginning of the division process is found to be a longitudinal splitting of the centroblepharoplast and the cone-shaped anterior portion of the body (pl. 8, figs. 33–36). This is followed by a splitting of the entire ectoplasmic surface of the body in the same plane, which is fundamentally longitudinal (pl. 9, fig. 51). This relation is maintained through the various stages of division to the early telophase (pl. 12, fig. 76). At this point, however, the activity of the motor organelles of the two attached daughter cells becomes operative in different directions, resulting in a change in the orientation of the two parts. With the continued opposing activities of the flagella the daughter flagellates are found attached to each other at the posterior regions only, giving an apparent transverse direction to the plane of division (pl. 12, fig. 79). This is apparent only and not the real direction which is fundamentally longitudinal. The morphological plane in which the chromosomes finally part at the metaphase coincides with that in which the highly organized neuromotor system is divided in plasmotomy. The plane of division of the chromomeres and chromosomes, and of the organized structures of the cytoplasm whose behavior at mitosis can be determined, is thus one and the same morphologically longitudinal plane.

RELATIONSHIPS

The relationships of these peculiar and highly evolved organisms has proven a source of some confusion. On the one hand ciliate affinities have been claimed for them, and, on the other, they have been listed as flagellates. Stein (1878), with the meager description given in Leidy's first paper in 1877 as his only basis of classification, correctly placed them among the flagellates. In this he has been followed by most later taxonomists. Leidy, however, with his fuller account of their structure in 1881, considered them intermediate between the gregarines and ciliates but more nearly related to the former. Kent (1882) followed this by placing them among the holotrichous ciliates in the faimly Trichonymphidae, a family he

formed for these parasites of the termites and included in it *Pyrsonema* and *Dinonympha*.

The flagellate affinities of *Trichonympha* were recognized by Grassi and Sandias (1893) who placed it in the family Lophomonadidae in the Flagellata. Bütschli (1889), however, reverts to the classification of Kent and recognized them as belonging to the ciliates. Senn (1900), with some doubt as to their actual position, placed them in the order Trichonymphida as an appendix to the Flagellata, but later (1911) allocated them in the Euflagellata. Hickson (1903) in Lankester's *Treatise on Zoology*, added the family Trichonymphidae as an appendix to the Ciliata. Doflein (1911) followed Senn in his classification of these puzzling forms, and, as had been earlier done by Bütschli, attributed the formation of the family Trichonymphidae to Leidy, overlooking the fact that Leidy nowhere attempted to classify the forms he described, while the family in question had been formed by Kent for these parasites of the termites.

The first complete systematic review of this subject is that given by Grassi (1911), in which he presents some changes in the previous taxonomic groupings. He formed a new order, the Hypermastigina, closely following the order Polymastigina in the Flagellata. This contained a single family, the Lophomonadidae Grassi. In this family he placed all the forms possessing many flagella, as *Trichonympha*, *Lophomonas*, and *Joenia*. Other changes that were made in the taxonomic position of other members of this group of parasites will be noted in a later paper discussing this subject.

In the genus *Trichonympha* he recognized two species, *T. agilis* Leidy and *T. minor* Grassi. The species described by Hartmann in 1910 as *T. hertwigi*, he rejects as defined by the describer, dividing the forms he has figured among three different genera, that is, the "young form" is referred to *Pyrsonympha*, and two new genera are created for the others. The "male" (Hartmann, 1910, pl. 28) he placed in the genus *Holomastigotoides* and the "female" (pl. 30) in the genus *Pseudotrichonympha*.

Poche, in 1913, added still further to the confusion already existing in this group by creating a new order, the Trichonymphida, which he placed in the Euflagellata. This contained four famliies, Dinenymphidae, Devescovinidae, Calonymphidae, and Trichonymphidae. The family Devescovinidae he formed for a single genus, *Devescovina* Foà, having but four flagella and in nowise related to the remainder of this group of many-flagellated organisms. This genus we consider

belongs in the order Polymastigina near the genus *Trichomonas*, to which its axostyle, parabasal body and four flagella ally it.

In a later paper we propose to give a fuller discussion of the systematic relations of the parasites of the termites and will here confine our attention to the genus *Trichonympha*, with the order and family to which it belongs.

The question of flagellate or ciliate relationships of *Trichonympha* is one the solution of which finds little difficulty in the light of facts concerning its morphology and division presented in this paper. We have already discussed some aspects of its relationships elsewhere (1919*c*) and will here only point out a few additional considerations. The relation between the motor organelles and the centroblepharoplast is distinctly flagellate in character. The nearest approach to this relationship among the ciliates is perhaps that found in the ciliate *Euplotes* (Yocom, 1918). Here the ciliary components of part of the cirri and the oral membranelles are bound together by their connection with the motorium. The latter structure, however, plays an entirely different rôle during division than does the centroblepharoplast of the flagellates. In *Euplotes* the motorium is passive or apparently disappears at the time of division and is formed anew in the daughter cells. In no case does it play an active part in mitosis. In the flagellates, on the other hand, this structure becomes the dominant, guiding figure in mitosis, dividing and acting as centrosomes in the formation of the mitotic spindle.

The occurrence of a highly differentiated ectoplasm is here correlated with the development of the complex myonemes, which in turn are probably directly correlated with the great increase in the number of flagella. A comparable degree of ectoplasmic differentiation is found in one other group of flagellates, the members of the subgenus *Pachydinium* in the genus *Gymnodinium* among the dinoflagellates (Kofoid and Swezy, 1919*d*), but without myonemes or flagella.

It is thus seen that in its morphology as well as division, *Trichonympha* possesses distinctly flagellate characteristics with none that are exclusively ciliate in character. The complexity of its structures is the result of a high degree of specialization and parallel evolution, and in no way connects it with the ciliates.

The possibility of these flagellates forming a connecting link between the Flagellata and the Ciliata, is also one for which we can find no adequate basis. Thus far no near relatives of *Trichonympha* have been found as free-living forms. They are confined exclusively

to the rôle of parasites or commensals of the termites and related insects. This fact in itself would throw them outside the line of evolution along which the present group of the presumably much earlier evolved, free-living ciliates were developed.

For these reasons we accept the allocation given by Grassi for these peculiar organisms, placing them among the true flagellates and, as shown by a study of the various stages of division as well as morphology, not far removed from certain members of the Polymastigina.

The utilization of the order Hypermastigina, proposed by Grassi (1911) for this group of flagellates possessing numerous flagella, is more appropriate as a descriptive term than either Trichonymphida Poche or Lophomonadina Lankester. It is further desirable as at once connoting its relation to the order Polymastigina near which it stands in the Flagellata.

For the family designation of the group to which *Trichonympha* belongs, we retain the term proposed by Kent (1882), Trichonymphidae. The remaining constituent members of this family we need not further specify at this time.

The genus *Trichonympha* contains three species previously described: *T. agilis* Leidy, from American termites; *T. Leidyi* Kent, from Tasmanian termites; and *T. minor* Grassi, from Italian termites. No figures have been given for the last two species, the description of both being imperfect without dimensions or other exact data. To these we add a fourth species, *T. campanula*.

Key to the Genus *Trichonympha*

Hypermastigina with flagella of various lengths disposed over about two-thirds of the surface of the body; nucleus submedian; specialized ectoplasm; complex neuromotor system; holozoic nutrition.

1. Large, 250μ–460μ 2
— Small, 100μ or less 3
2. Flagella of three distinct lengths, posterior endoplasm not separated from anterior region by distinct line ..... **campanula** sp. nov.
3. Flagella short, almost cilia-like, elongate or pyriform body ..... **leidyi** Kent
— Distinct line separating two regions of endoplasm, body constricted at the point of separation, cilia long ..... **agilis** Leidy
— Small form, flagella short, lines separating two regions of endoplasm long, crossing each other behind nucleus ..... **minor** Grassi

SUMMARY

1. This organism has a highly specialized flagellate type of structure with a highly developed neuromotor system, the centro blepharoplast connected by a complex system of oblique fibers with the numerous flagella which cover two-thirds of the surface of the body. Besides these fibers the ectoplasm contains an alveolar layer and one of transverse myonemes. Immediately below it in the endoplasm are the longitudinal myonemes.

2. The nucleus is submedian in position and part of its chromatin contents is permanently separated as a "heterochromosome" contained within a small vesicle.

3. Nutrition is holozoic but its method of feeding is unknown. No cytostome is present. The endoplasm is divided into two regions, anterior and posterior. The latter region, which is covered only by a thin pellicle and not the thick ectoplasm of the remainder of the body, is usually filled with food particles. These are entirely absent from the anterior region of endoplasm.

4. The body rounds up at the time of division and the centroblepharoplast divides, forming a paradesmose, the entire ectoplasm splitting into two parts with it. These act as the centrosome in the succeeding mitotic figures, the spindle fibers arising from the ends of the paradesmose or the centroblepharoplasts.

5. Precocious splitting of the chromosomes takes place previous to the prophase, forming fifty-two, V-shaped threads. In a pseudotelosynapsis this number is reduced to twenty-six. These part on the spindle along the line of the original split.

6. Division of the chromosomes as well as the body is fundamentally longitudinal.

7. *Trichonympha campanula* is fundamentally flagellate in its morphology and method of division and is in nowise related to the ciliates. It shows a high degree of specialization and development of its neuromotor system which is the most complex one thus far described among the Protozoa.

8. It is a member of the family Trichonymphidae Kent in the order Hypermastigina Grassi. This order stands near the Polymastigina, to the members of which *Trichonympha* is nearly related both morphologically and in its development.

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LITERATURE CITED

Boeck, W.

1917. Mitosis in *Giardia microti*. Univ. Calif. Publ. Zool., **18**, 1–26, pl. 1.

Bütschli, O.

1889. Trichonymphidae Leidy 1877 emend., *in* Protozoa *in* Bronn, Klass. u. Ordn. des Thierreichs, **1**, part 3, pp. 1774–78, pl. 76, figs. 1, 3–6.

Calkins, G. N.

1899. Mitosis in *Noctiluca miliaris* and its bearing on the nuclear relations of the Protozoa and Metazoa. Jour. Morph., **15**, 715–772, pls. 40–42.

Chambers, R.

1917. Microdissections studies. II, The cell aster; a reversible gelation phenomenon. Jour. Exp. Zool., **23**, 483–504, pl. 1.

Doflein, F.

1911. Lehrbuch der Protozoenkunde. Eine Darstellung der Naturgeschichte der Protozoen mit besonderer Berücksichtigung der parasitischen und pathogenen Formen. (Ed. 3, Jena, Fischer), xii + 1043 pp., 951 figs. in text.

Foà, A.

1904. Richerchi sulla riproduzione dei Flagellati. II, Processo di divisione delle *Triconymphi*. Rend. R. Accad. dei Lincei, Cl. Sci. Fis. Mat. e Nat., Rome, (5), **13**, 618–620, 5 figs. in text.

Grassi, B.

1911. Intorno ai Protozoi dei Termitidi. *Ibid.*, (5), **20**, 725–741.

Grassi, B., and Sandias, A.

1893. Constituzione e sviluppo della societa dei Termitidi. (Catania, Galatola), pp. 1–151, pls. 1–5.

Hartmann, M.

1910. Bau und Entwicklung der Trichonymphiden (*Trichonympha hertwigi*). Festschr. z. Hertwigs, pp. 349–392, pls. 27–30, 3 figs. in text.

Hickson, S. J.

1903. The Infusoria or corticate Heterokaryota, *in* Lankester, Treatise on Zoology, **1**, sec. 2, pp. 361–426, 97 figs. in text.

Kent, S.

1880–82. A manual of Infusoria. (London, Bogue), **1**, **2**, 1–913, pls. 1–50.

1884. Notes on the infusorial parasites of the Tasmanian white ant. Papers and Proc. Roy. Soc., Tasmania, **1884**, 270–273.

Kofoid, C. A., and Christiansen, E. B.

1915. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–34, pls. 5–8, 1 fig. in text.

Kofoid, C. A., and Swezy, O.

1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Am. Acad. Arts Sci., **51**, 289–378, pls. 1–8, 7 figs. in text.

1919*a*. Parasites of the termites, I. On *Streblomastix strix*, a polymastigote flagellate with a linear plasmodial phase. Univ. Calif. Publ. Zool., **20**, 1–20, pls. 1, 2, 1 fig. in text.

1919*b*. Studies on the parasites of the termites, II. On *Trichomitus termitidis*, a polymastigote flagellate with a highly developed neuromotor system. *Ibid.*, **20**, 21–40, pls. 3, 4, 2 figs. in text.

1919*c*. Flagellate affinities of *Trichonympha*. Proc. Nat. Acad. Sci., **5**, 9–16, figs. 1–10.

1919*d*. A monograph of the unarmored Dinoflagellata. Mem. Univ. Calif. In press.

Leidy, J.
1877. On intestinal parasites of *Termes flavipes*. Proc. Acad. Nat. Sci. Philadelphia, **1877**, 146–149.
1881. The parasites of the termites. Jour. Acad. Nat. Sci. Philadelphia, (2), **8**, 425–450, pls. 51, 52.

Poche, F.
1913. Das System der Protozoa. Arch. Prot., **30**, 125–321, 1 fig. in text.

Porter, J. F.
1897. *Trichonympha* and other parasites of *Termes flavipes*. Bull. Mus. Comp. Zool., Cambridge, **31**, 48–68, pls. 1–6.

Senn, G.
1900. Flagellata, *in* Engler and Prantl, Die natürlichen Pflanzenfamilien. (Leipzig, Englemann), **1**, part 1, a, pp. 93–192, text figs. 63–140.
1911. *Oxyrrhis, Nephroselmis* und einige Euflagellaten. Zeitschr. wiss. Zool., **97**, 605–672, pls. 30, 31, 8 figs. in text.

Sharp, R. G.
1914. *Diplodinium ecaudatum* with an account of its neuromotor apparatus. Univ. Calif. Publ. Zool., **13**, 43–122, pls. 3–7, 4 figs. in text.

Stein, F.
1878. Die Organismus der Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. Der Organismus der Infusionsthiere. 3. Abth., Die Naturgeschichte der Flagellaten oder Geisselinfusorien. 1. Hälfte, Den noch nicht abgeschlossenen allgemeinen Thiel nebst Erklärung der sämmtlichen Abbildungen enthaltend, (Englemann, Leipzig), pp. 1–154, pls. 1–24.

Wilson, E. B.
1911. The sex chromosomes. Arch. mikr. Anat., **77**, 249–271, 5 figs. in text.
1913. A chromatoid body simulating an accessory chromosome in *Pentatoma*. Biol. Bull., **24**, 392–410, pls. 1–3.

Yocom, H. B.
1918. The neuromotor apparatus of *Euplotes patella* Ehrbg. Univ. Calif. Publ. Zool., **18**, 337–396, pls. 14–16, 1 fig. in text.

EXPLANATION OF PLATES

All figures of *Trichonympha campanula* sp. nov. from *Termopsis angusticollis* Walker, stained with iron haematoxylin and drawn with camera lucida. Magnification 800, unless otherwise stated.

PLATE 5

Fig. 1. Normal trophozoite showing the three zones of flagella, surface ridges of the body, and centroblepharoplast. × 300.

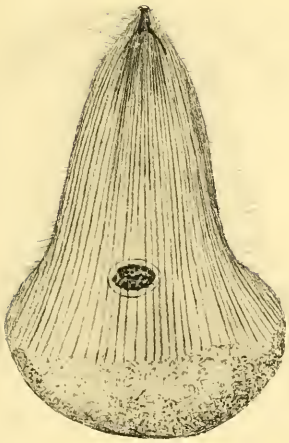
Fig. 2. Optical section of anterior part of the body, showing the differentiations of ectoplasm and endoplasm with the longitudinal myonemes in the latter, and part of the centroblepharoplast. × 300.

Fig. 3. Surface view of anterior end showing surface ridges, the cuplike depression with end of centroblepharoplast at its base and its covering operculum. Internally the ectoplasm and endoplasm may be seen with the vacuoles near the base of the centroblepharoplast.

Fig. 4. Semidiagrammatic cross-section of body in the region of the nucleus, showing origin of flagella from crests of surface ridges, ectoplasm, endoplasm, and the longitudinal myonemes in the latter. × 300.

Fig. 5. Optical section of anterior portion of the body showing striate appearance of ectoplasm, with its different layers, and the circular vacuole at the base of the centroblepharoplast. × 300.

Fig. 6. Optical section of entire body showing extent of differentiated ectoplasm and the two regions of endoplasm. Dark granules are probably remnants of ingested bacteria. × 300.



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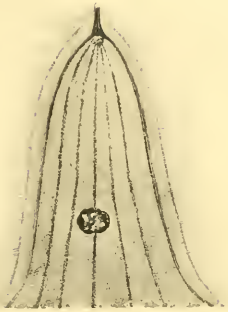

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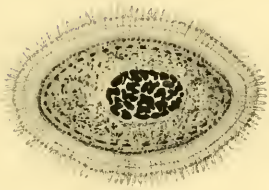

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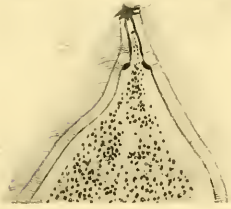

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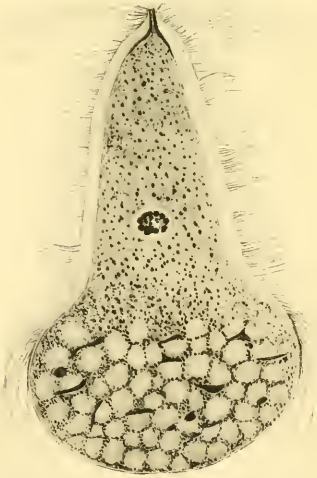

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PLATE 6

Fig. 7. Vertical view of centroblepharoplast complex in rounded-up form. Cuplike depression and operculum have disappeared. Oblique fibers radiating out from lobes of centroblepharoplast.

Fig. 8. Cross-section of distorted individual showing relative extent of endoplasm and ectoplasm. × 300.

Fig. 9. Lateral view of anterior end showing centroblepharoplast complex, oblique fibers and alveolar layer.

Fig. 10. Centroblepharoplast complex of individual in early prophase. Operculum and depression have disappeared, end of centroblepharoplast drawn out to a point.

Fig. 11. Vegetative nucleus showing the central region of chromatin granules, the heterochromosome and its vesicle, the alveolar zone and the outer, granular region.

Fig. 12. Cross-section of anterior end of body showing the centroblepharoplast, myonemes and surface ridges.

Fig. 13. Vertical view of centroblepharoplast complex of the early prophase stage.

Fig. 14. Vegetative nucleus showing the chromatin-encrusted network. Other parts as in figure 11.

Figs. 15–22. Nuclei of the early prophase stages in individuals which present no other sign of division.

Fig. 15. Early prophase; alveolar zone has disappeared, chromatin moving out from the granules along the threads of the network.

Fig. 16. Later stage of the same. Note size of heterochromosome and its vesicle.

Fig. 17. Formation of chromosomes; vesicle surrounding heterochromosome has disappeared.

Fig. 18. Appearance of distinct, paired chromosomes.

Figs. 19–22. Various figures of fully formed chromosomes.

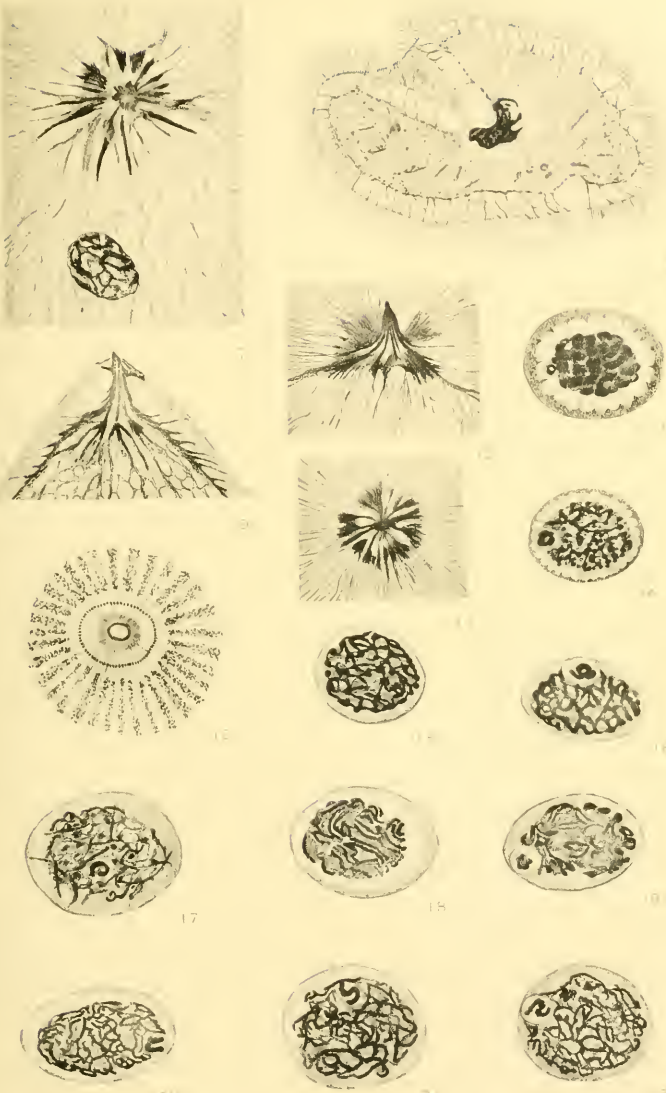
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PLATE 7

Fig. 23. Chromosomes appearing in the nucleus before other signs of division can be detected in the body. Figures 25–29 were drawn from similar individuals. × 300.

Fig. 24. Rounding up of the body preparatory to division; part of the centroblepharoplast complex and alveolar layer shown. × 300.

Fig. 25.—Longitudinal division of the chromosomes.

Fig. 26.—Breaking up of the chromatin-encrusted network beginning at one side of the nucleus.

Fig. 27. Network thickly encrusted with chromatin showing free ends.

Fig. 28. Later stage of the same with the threads beginning to split.

Fig. 29. Chromosome formation nearly completed.

Fig. 30. Beginning of division of the centroblepharoplast complex. Note the spindle-shaped areas of endoplasm appearing between split.

Fig. 31. Optical section of rounded-up individual of the prophase period. × 300.

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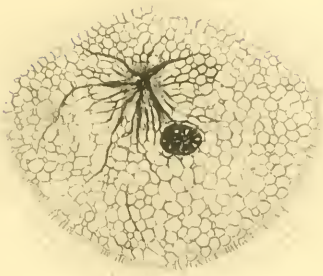

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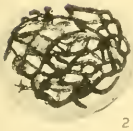

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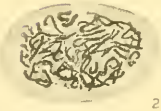

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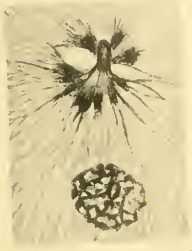

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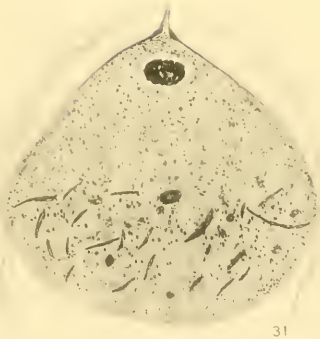

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PLATE 8

Fig. 32. Prophase with division of the centroblepharoplast completed in the tubular part but no split yet appearing in the ectoplasmic structures. An unusual figure. × 300.

Fig. 33. Splitting of the centroblepharoplast and the formation of the paradesmose between the bases as they separate.

Fig. 34. A later stage of the same with the halves forming new tubes connected at the tip. Note development of spines at tip.

Fig. 35. An earlier stage of the same showing the split beginning at the base, with the ectoplasmic structures drawing apart.

Fig. 36. Splitting completed; paradesmose elongates as the new centroblepharoplasts move apart.

Fig. 37. Vertical view of an early stage of splitting of centroblepharoplast.

Fig. 38. Vertical view of individual in prophase with the divided centroblepharoplasts connected by paradesmose. Part of oblique fibers and alveolar layer shown. Note aster arrangement of alveoli aronnd centroblepharoplasts. × 300.

Fig. 39. Prophase nucleus showing the breaking up of the chromatin-encrusted network.

Fig. 40. Paradesmose, centroblepharoplasts and their related fibers in the prophase, with the nucleus elongating preparatory to formation of mitotic spindle.

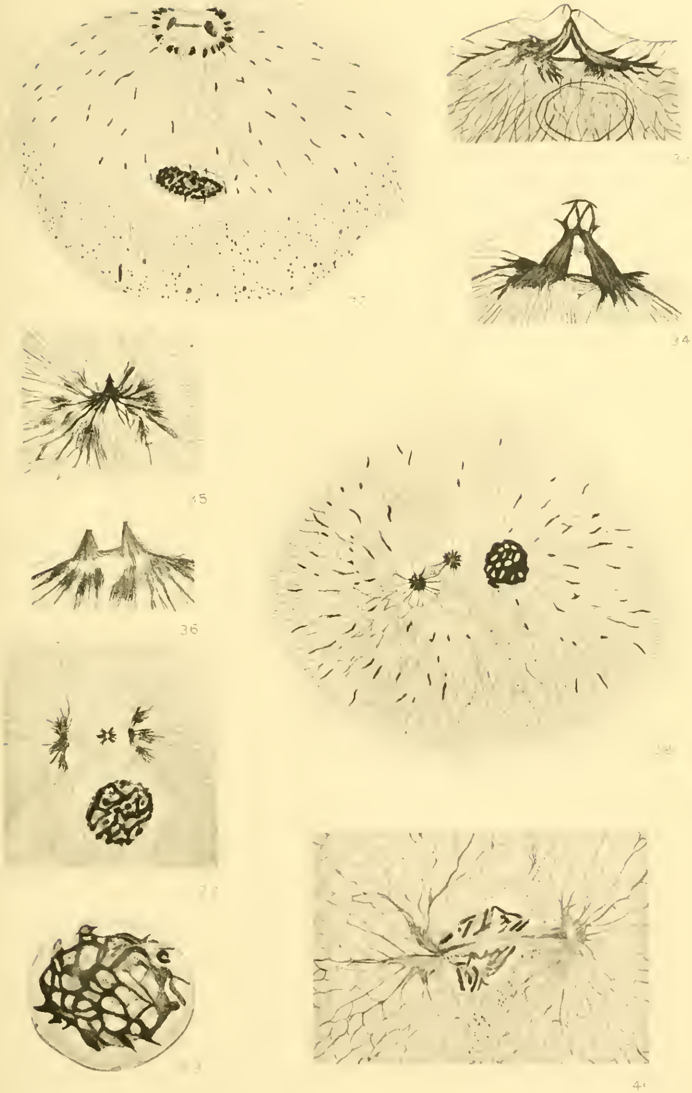
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PLATE 9

Figs. 41, 42. Two views of the same figure showing different structures. 41. The paradesmose, centroblepharoplast and oblique fibers. Immediately below and intermingled with these are the structures shown in 42: prophase nucleus and alveolar layer.

Fig. 43. Nucleus becoming closely attached to the paradesmose.

Fig. 44. Nucleus showing fifty-two chromosomes with marked chromomere structure. × 1250.

Fig. 45. Prophase nucleus in the tangled-skein stage.

Fig. 46. Prophase nucleus with looped chromosomes.

Fig. 47. Same stage. In both figures heterochromosome remains isolated.

Fig. 48. Beginning of the tangled-skein stage (?); chromosomes at one side of nucleus stain deeper than those on the other.

Fig. 49. Marked tendency of chromosomes to assume paired arrangement.

Fig. 50. Tangled-skein stage.

Fig. 51. Individual in prophase showing completion of division of ectoplasmic structures. Only a few of the surface ridges are indicated. × 300.

Fig. 51*a*. Enlarged view of nucleus of last figure. Chromosome number reduced to 26.

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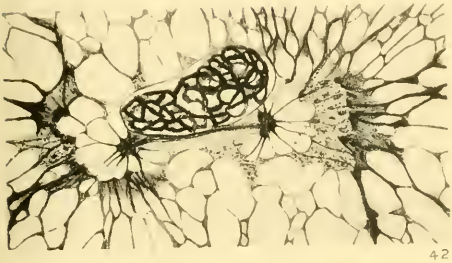

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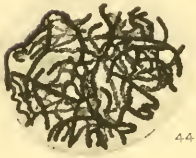

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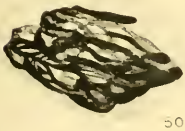

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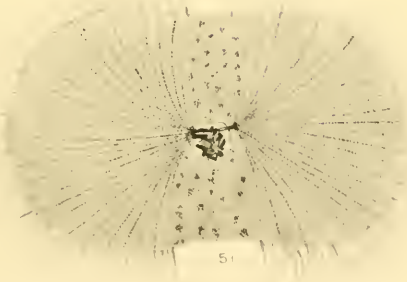

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PLATE 10

Nuclei on this and the following plates have the reduced number of chromosomes. In some figures the paradesmose is shown in position, i.e., above the nucleus. In others it is omitted or only indicated by faint lines to secure clearness.

Fig. 52. Late prophase; nucleus elongated and attached to the paradesmose.

Fig. 53. Same stage; tubular part of paradesmose omitted.

Fig. 54. Chromosome arrangement previous to spindle formation.

Fig. 55. Spindle fibers extending from centroblepharoplasts through nuclear membrane and attached to chromosomes.

Fig. 56. Showing process of unfolding of chromosomes in the metaphase. Heterochromosome distinct. Vertical view, with paradesmose uppermost and outside nuclear membrane.

Fig. 57. Lateral view of same stage with paradesmose above the nucleus.

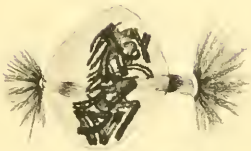
Fig. 58. Same stage viewed from below, i.e., the interior of the body. Paradesmose is on opposite side of nucleus.

Fig. 59. Metaphase; paradesmose partly imbedded within the nucleus.

Fig. 60. Final division of the chromosomes; heterochromosome distinct and still undivided. Entire nucleus elongating.

Fig. 61. Early anaphase; heterochromosome divided.

52



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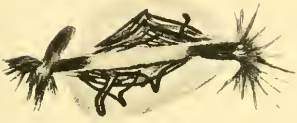

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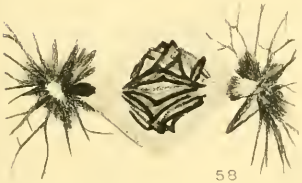

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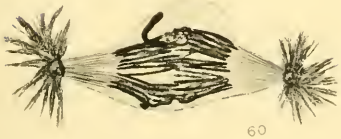

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PLATE 11

Fig. 62. Anaphase; heterochromosome divided without apparent attachment to spindle fibers.

Fig. 63. Unusual appearance of chromatin granules strung along the spindle fibers. × 1250.

Fig. 64. Anaphase; heterochromosome dividing.

Fig. 65. Telophase of nucleus; constriction of the nuclear membrane. × 1250.

Fig. 66. Daughter nucleus with division completed.

Fig. 67. Telophase with nuclear constriction advancing. Spindle fibers disappearing at one pole, still intact at the other. × 1250.

Fig. 68. Telophase showing surface ridges of the body. Centroblepharoplasts still connected by the paradesmose. × 300.

Fig. 69. Telophase of nucleus.

Fig. 70. Daughter nucleus after constriction and prior to rounding up.

Fig. 71. Greatly elongated nuclear band still connecting daughter nuclei. × 300.

Fig. 72. Completion of nuclear division with paradesmose still persisting.

Figs. 73, 74. Sister nuclei recently divided.

Fig. 75. Completion of nuclear division. Paradesmose beginning to fade out.

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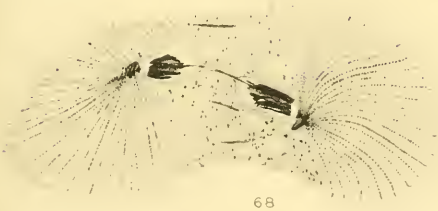

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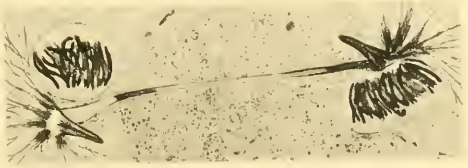


PLATE 12

Fig. 76. Late telophase; nuclei have become reorganized; alveolar layer shown. × 300.

Fig. 77. Nucleus at the beginning of reorganization.

Fig. 78. Later stage showing chromosomes forming a coarse network.

Fig. 79. Plasmotomy; all ectoplasmic structure except oblique fibers are omitted. × 300.

Fig. 80. Optical section of individual shown in figure 76 at the point marked by the arrow. Note basal granules and basal portion of flagella.

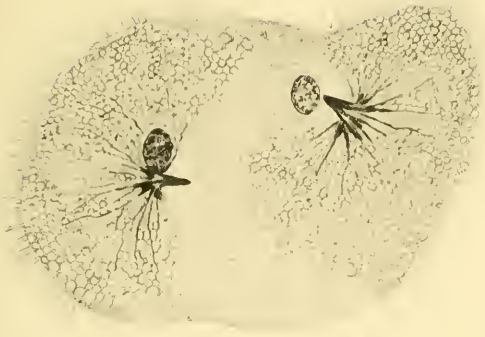
Fig. 81. Reorganization process of nucleus.

Fig. 82. Early stage of the same process.

Fig. 83. Later stage showing the vesicle forming around the heterochromosome.

Fig. 84. After completion of plasmotomy. Nuclear reorganization not yet begun. × 300.

Fig. 85. Somatella prior to plasmotomy. Note lack of synchronism in development of ectoplasmic structures. × 300.



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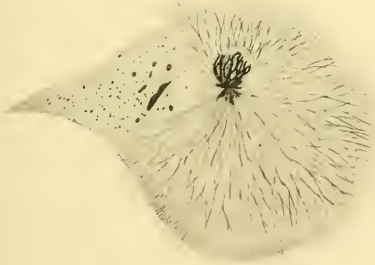

89

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BY
CHARLES ATWOOD KOFOID AND OLIVE SWEZY

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CONTENTS

	PAGE
Introduction	100
Morphology	100
Shape and size	101
Neuromotor system	102
Ectoplasmic structures	102
Surface ridges	103
Locomotor organelles	103
Oblique fibers	103
Centroblepharoplast	104
Alveolar layer	105
Endoplasmic structures	105
Longitudinal myonemes	105
Nucleus	106
Binary fission	107
Division of ectoplasmic structures	107
Mitosis	108
Prophase	108
Metaphase	109
Anaphase	109
Telophase	110
Relationships	110
Summary	111
Literature cited	113
Explanation of plates	114

INTRODUCTION

In our previous studies on the parasites of the termite, *Termopsis angusticollis* Walker, we have described three of the curious and highly interesting protozoans in this faunal complex. These are the flagellates *Streblomastix strix* (1919*a*), *Trichomitus termitidis* (1919*b*), and *Trichonympha campanula* (1919*c*). A fourth member of this group remains to be noticed. This flagellate is closely akin to *Trichonympha campanula* yet presents some striking differences which, in our opinion, give it a position generically distinct from that species. We therefore propose for it the name *Leidyopsis sphaerica* gen. nov., sp. nov., naming it in honor of the pioneer investigator of this group, the American naturalist Dr. Joseph Leidy.

It is less abundant than are the other members of this association of termite parasites. It is found in the lumen of the intestinal tract and is in no case attached to the walls. In this location, as also in its activities, it resembles *Trichonympha campanula*. The mass of flagella attached to the anterior portion of the body stream backward, partly clothing its rotundity. In the living animal the action of the flagella is similar to that of its larger relative. Waves of contraction pass from the proximal to the distal ends of the flagella without cessation. Owing to the differences in the shape of the body its anterior end is slightly less mobile than is the case in *Trichonympha campanula*. Its rate of progression is also somewhat slower, as though impeded by the rotundity of the body in moving through the seething mass of organisms found in the intestinal tract.

MORPHOLOGY

In its morphology *Leidyopsis* presents a type of structure which is as highly differentiated as that found in *Trichonympha*. It differs from it mainly in the extent and distribution of the specialized structures as compared with the remainder of the body. These differences will be pointed out more specifically below.

The outstanding feature of its morphology, next to its abundant supply of flagella, is its neuromotor system, which is of the trichonymphid type (Kofoid and Swezy, 1918*c*). Correlated with this are the ectoplasmic differentiations, while the remainder of the body presents no structural features which mark an advance over that of the simpler flagellates. The evolutionary development of organelles

in *Leidyopsis* is thus marked only in its neuromotor system, while the remainder of the body still shows a low degree of development. Its mode of nutrition is holozoic, as shown by the food particles in the endoplasm, yet no cytostome or other organelles for food taking are present.

Shape and Size

In size *Leidyopsis sphaerica* presents less variation than has been found in the three other flagellates previously described from the same habitat. This is probably due, however, to the paucity of our

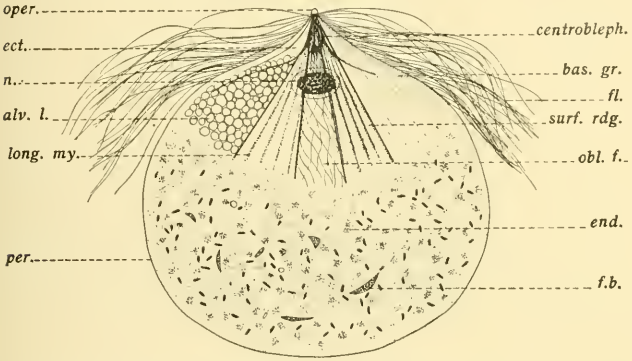

Fig. A. Diagrammatic figure of *Leidyopsis sphaerica* gen. nov., sp. nov. Ectoplasm drawn in sections to show structure of its different parts.

Abbreviations: *alv. l.*, alveolar layer; *bas. gr.*, basal granules; *centrobleph.*, centroblepharoplast; *ect.*, ectoplasm; *end.*, endoplasm; *f. b.*, food bodies; *fl.*, flagella; *long. my.*, longitudinal myonemes; *n.*, nucleus; *obl. f.*, oblique fibers; *oper.*, operculum; *per.*, periplast; *sur. rdg.*, surface ridges. × 400.

material, as a larger number of individuals would present room for greater variations. It is nearly spherical in shape, its length only a few microns greater than its width, due to the cone-shaped projection of the anterior end of the body. The length varies from 165 to 190μ and the width 160 to 185μ. Figure 1 of plate 13 is that of an individual 182μ in length and 176μ in breadth. These proportions vary considerably, particularly in stained material where the body becomes flattened and relative proportions almost entirely lost. Measurements are of value only when made from the living organism.

The body is perfectly symmetrical in outline, a condition found in only a few protozoans. The shape is that of a sphere with the

anterior end drawn out into a short cone-shaped projection. This is terminated by a small, transparent, caplike structure, the operculum (fig. A, *oper.*), which covers a cup-shaped depression (pl. 13, fig. 10). This is similar to the structure of the anterior end of *Trichonympha campanula*. No evidences have been found which would indicate the function of this peculiar formation. It is the only part of the surface of the body bearing any resemblance to a cytostomal opening into the endoplasm, yet we have no evidence that it is used for that purpose.

The surface of the anterior and sometimes even of the middle portions of the body is marked by longitudinal ridges which extend from the operculum posteriorly (fig. A, *surf. rdg.*). In the posterior regions these are entirely lacking, the surface having a smooth, unbroken outline.

Neuromotor System

The neuromotor system of *Leidyopsis* resembles that of *Trichonympha*. It consists of a highly developed, anteriorly located centroblepharoplast, numerous flagella, and two sets of fibers, the oblique fibers in the ectoplasm and the longitudinal myonemes in the endoplasm. The transverse myonemes of *Trichonympha* are not found in this form and the others are not so well developed as are those of that genus.

Ectoplasmic Structures

The division of the body into ectoplasm and endoplasm is clearly marked only in a narrow zone surrounding the cone-shaped anterior end of the body. Here it presents the three distinct layers so striking in the ectoplasm of *Trichonympha*, but these soon fade out, though the myonemes and surface ridges can be traced farther posteriorly, the latter usually having a length of one-third to one-half that of the body. Anteriorly the ectoplasm is thick around the base of the cone, becoming thin posteriorly until it merges into the thin periplast of the middle and posterior regions of the body (fig. A). It is composed of an outer layer of surface ridges (fig. A, *surf. rdg.*), an alveolar layer (*alv. l.*), and a narrow, inner ectoplasmic layer. These are traversed by the oblique fibers and also contain the basal granules of the flagella. These separate structures will now be discussed in detail.

Surface Ridges: The outer layer of ectoplasm is raised in narrow longitudinal ridges (fig. A, *surf. rdg.*) from the crests of which spring the flagella. The ridges extend from the base of the operculum at the anterior end posteriorly for about one-third to one-half the length of the body. Anteriorly they are very narrow and placed close together, the ends forming an irregular, wavy line around the base of the operculum (pl. 13, fig. 10). Posteriorly they spread out in fan-shaped or radiating lines, increasing in number by the interposition of new ridges. Their course is longitudinal without spiral twisting, and when the limits of the differentiated ectoplasm are reached they disappear in the thin periplast of that region.

Locomotor Organelles: These are confined to the anterior third of the body, and consist of long flagella, approximately equal in length and arising in longitudinal rows from the crests of the surface ridges. They are most numerous anteriorly on the cone-shaped portion of the body, where they form a dense mass which retains the dark color of iron haematoxylin. A single flagellum alone does not show the stain nor do the ends which stream out from this mass. Collectively, however, they are usually stained so darkly as to obscure the nucleus and other structures beneath them. The spreading apart of the surface ridges distally results in a distinct thinning of the coating of flagella behind the narrowed anterior end of the body.

Each flagellum arises from a minute basal granule below the surface ridges, passes up through the ridge and leaves the crests as a single thread. Each basal granule is connected with a minute fibril from the oblique fibers, apparently in the same manner as in *Trichonympha campanula*. These fibrils and basal portions of the flagella give a finely striate appearance to the layers of ectoplasm through which they pass.

The flagella stream outward and backward over the surface of the body and in the living flagellate are in constant motion.

Oblique Fibers: These are not so prominent in *Leidyopsis* as in *Trichonympha*, owing partly to their smaller extent, and partly to the difficulty of differentiating them from the mass of dark flagella. When the flagella have been sufficiently destained to allow observation of the structures beneath them, the latter have also completely lost their color, and only the most careful focusing enables the observer to distinguish between the flagella and the equally slender oblique fibers immediately beneath.

The oblique fibers form an integral part of the centroblepharoplast

complex. They arise from its basal lobes as branches which break up into the minute, threadlike oblique fibers. These follow an oblique course posteriorly, continually giving off branches, part of which go to the basal granules of the flagella and the remainder cross and intercross in an irregular anastomosing network through the ectoplasm of the body (fig. A, *ob. f.*). Near the distal limits of the ectoplasmic zone these fibers fade out and disappear.

In their structure they are homogenous and not granular, and their width is about equal to that of the flagella. The smaller branches leading out to the basal granules are more slender. At the anterior end, near the basal lobes of the centroblepharoplast, they take a darker stain than is the case farther posteriorly, though this may be largely due to their slightly greater thickness in the posterior region.

Centroblepharoplast: The oblique fibers are intimately related to the centroblepharoplast. This organelle in *Leidyopsis* differs but little if any from that of *Trichonympha*. It forms a tubular or rod-shaped structure in the pointed anterior end of the body (fig. A, *centrobleph.;* pl. 13, figs. 1, 3), from the base of which stream out the oblique fibers.

The tubular part of the centroblepharoplast is composed of a number of rods or strands which extend to the base of the cuplike depression at the anterior end of the cone-shaped portion of the body. Here the ends are joined together by a circular band of darkly staining material, the center of which is occupied by the core of endoplasm (pl. 13, figs. 2, 10, 13). The tube extends posteriorly to near the base of the cone where it expands into a collar-like structure composed of several large, irregular lobes, which may sometimes be united into a solid band encircling the base of the tube (pl. 13, fig. 3). Distally these lobes fray out into the oblique fibers.

The center of the tube is occupied by a slender core of endoplasm continuous with the endoplasm of the body. This extends up to the base of the small, cup-shaped depression, where it may be seen as a light area within a dark ring (pl. 13, fig. 10).

The tubular part of the centroblepharoplast complex may present some modifications, such as the formation of short, spinelike processes extending out from the tip (fig. 3) or along its sides (fig. 1). At the time of division the cuplike depression and operculum disappear, leaving the tip of the centroblepharoplast complex exposed at the surface. The entire structure takes a deep stain with iron haematoxylin, making it a conspicuous part of the body in stained preparations.

Alveolar Layer: This is closely associated with the oblique fibers, occupying the same region of ectoplasm. It is difficult to demonstrate and sometimes appears to be entirely lacking. This, however, seems to be due to the small extent of the ectoplasmic layer which it covers in those cases. In only a few specimens does it extend as far posteriorly as do the other ectoplasmic structures. It seems to be the first part to disappear in the thinning of the ectoplasm, which takes place a short distance behind the base of the cone.

The alveoli are most prominent at the time of division and appear in surface view as small, colorless spheres, varying considerably in size, and not closely packed together (pl. 14, fig. 24). In the ordinary trophozoite the arrangement is more compact, with the individual alveoli of smaller size.

Endoplasmic Structures

The endoplasm may be divided into two regions, anterior and posterior, but with no definite boundary line separating them. The distinction between the two portions is rather less pronounced in this form than in the different species of *Trichonympha*.

The anterior part is relatively small, extending but a short distance behind the nucleus (pl. 13, fig. 1). It is granular, without alveoli or food inclusions. The part immediately surrounding the nucleus is usually more dense than the remaining portion, evidently the result of greater metabolic activity.

The posterior region of endoplasm is coarsely vacuolate in structure and is often abundantly filled with food bodies (fig. A, *f. b.*). These are sometimes found enclosed in food vacuoles but more frequently are found lying free in the plasma. They consist of particles of wood, small flagellates, bacteria, or other small bodies that may be present in the intestinal contents. The method of ingestion of these is entirely unknown. No cytostome is present unless it is possible for the cup-shaped depression at the anterior tip of the body to assume that function. Neither the feeding reactions nor defecation of *Leidyopsis* have been observed.

Longitudinal Myonemes: These myonemes are found in the outer zone of endoplasm, immediately beneath the ectoplasm of the anterior part of the body, and coextensive with it. They are granular in structure and form straight, longitudinal lines radiating out from the region of the centroblepharoplast (fig. A, *long. my.*). Their connection with that structure, if any exists, could not be clearly detected.

Near the distal limits of the ectoplasmic differentiation the longitudinal myonemes fade out in the endoplasm, without showing distinct attachment areas. Their function here, as in *Trichonympha*, seems to be concerned with the mobility of the anterior tip of the body. The globular form of *Leidyopsis* as compared with the elongate one of *Trichonympha* allows for much less activity, yet the same type of sidewise movements, though greatly restricted, may be observed in the living, active flagellate.

Nucleus: The nucleus is found in the anterior part of the body, a short distance posterior to the centroblepharoplast (fig. A, *n.*). It is a rotund ellipsoid, with its longer axis, which lies in the transverse plane of the body, exceeding its shorter axis by nearly a third of its own length. No rhizoplast could be detected between it and the centroblepharoplast.

The structure of the nucleus is like that of *Trichonympha campanula*. The membrane is thin and overlies a narrow granular zone, beneath which is an alveolar zone. These alveoli are rounded outwardly where they abut upon the granular zone, with the remaining faeets closely pressed together. The alveoli are fairly uniform in size, yet in stages which show distinct chromosome changes prior to the prophase of division, great modifications may often be noticed. The alveoli may be fewer in number and larger in size. This may be the result of a breaking down of the walls between several adjacent alveoli, since in the early prophase their disappearance is usually complete.

The remainder of the nucleus inside the alveolar zone is occupied by an irregular linin network in which loose ends may often be detected. The network is usually encrusted with chromatin, with large granules of the same substance at the nodes. The disposition of the chromatin, however, varies considerably in different individuals and is probably conditioned by the chromosome cycle, which here apparently follows a course similar to that previously outlined for *Trichonympha campanula* (Kofoid and Swezy, 1919*c*). The nuclei shown in figures 5 to 8 on plate 13, give different phases of nuclear structures in individuals which do not yet give other indications of the approach of division. In these nuclei the network progressively breaks up, the chromatin moving out from the granules along the threads which become thicker and split lengthwise (fig. 6). From these threads the definitive chromosomes are produced.

The nucleus of *Leidyopsis* is further distinguished by the presence of a small, coiled chromatin rod similar to the heterochromosome of *Trichonympha campanula*. This is isolated in a small, clear vesicle at one side of the central chromatin mass and partially imbedded within it.

BINARY FISSION

The process of binary fission in *Leidyopsis sphacrica* shows a close similarity to the various phases of binary fission in *Trichonympha campanula*. It is characterized by the longitudinal division of the centroblepharoplast complex and of the ectoplasmic structures, the formation of a paradesmose, precocious, longitudinal splitting of the chromosomes and pseudosynapsis. Owing to the scanty numbers of these flagellates which have been present in any one host, we cannot present the full details of the different stages. The close similarity between the phases that we have secured and those of the other species, however, would seem to suggest that the remaining stages of *Leidyopsis* are also similar. In the following discussion, therefore, the different phases are interpreted in the light of our fuller knowledge of the mitotic phenomena of *Trichonympha*.

Division of Ectoplasmic Structures

The first evidences of division in the extranuclear structures of the body are found in the centroblepharoplast. This structure divides, beginning at the base and splitting longitudinally to the tip. The basal masses become separated into two equal parts (pl. 13, figs. 12, 13), taking with them their attached fibers and motor organelles. This divides the entire ectoplasmic layer, leaving a constantly increasing strip of endoplasm between them as they move apart. As the two halves of the centroblepharoplast separate, a broad band, the paradesmose, is formed between them (pl. 13, figs. 15, 16). This is attached directly to the bases, leaving the tubular portions of the centroblepharoplasts standing out from it at right angles, or nearly so. In figures 15 and 16 these parts are omitted, as the structures are viewed from the posterior end of the organism and show the relations of the paradesmose and the basal lobes of the centroblepharoplasts. Here, as in *Trichonympha*, with the final separation of the tips of the centroblepharoplast, each half develops into a tube similar to the original structure. This seems to take place immediately after separation of the halves.

The further course of division of the ectoplasmic structures shows a continued separation of the two portions until they come to lie on opposite ends of the organism, connected by the spindle and paradesmose (pl. 14, fig. 17). The relatively large portion of the body which is not covered with ectoplasmic structures and flagella, renders this separation very conspicuous in these stages. The completion of these structures for each daughter cell is partly the result of new outgrowths and partly the readjustments of those derived from the parent cell. At the time plasmotomy occurs these may be completed or may still be in the process of formation (pl. 14, figs. 24, 25).

Mitosis

The first evidences of the approach of mitosis may be looked for in the nucleus. How early these appear cannot be stated, but, as in the case of *Trichonympha*, the relative abundance of individuals showing nuclear organization leading to the appearance of distinct chromosomes, would suggest that it begins soon after the completion of a previous division period.

Prophase: The chromatin of the nucleus may be disposed in large granules (pl. 13, fig. 2) or in smaller granules with the network connecting them thickly encrusted with chromatin (fig. 4). In the change from one condition to the other there seems to be a reduction in the amount of chromatin. The nucleus, however, becomes enlarged hence the reduction may be more apparent than real. The chromatin of the granules continues to move out along the network until the threads of the latter have become greatly thickened and the granules have disappeared (pl. 13, fig. 5). This produces a coarse, heavy network in which many free ends may be detected (fig. 12). These are most frequently found at one side of the nucleus, with the opposite side staining more densely and presenting an unbroken outline. A further disintegration of the network is shown in figure 9 of plate 13. Here its component parts are forming threads which begin to take on the appearance of chromosomes on one side of the nucleus, while the other still retains its network formation.

The threads thus formed split longitudinally (pl. 13, fig. 6), while at the same time becoming thicker, with the chromatin arranged in distinct chromosomes. The outer alveolar zone of the nucleus disappears, though this may frequently occur at an earlier stage. The chromosomes, which at first are straight or irregularly twisted or bent

(pl. 13, figs. 7, 8, 11), gradually assume a V-shaped form, and the cloudy appearance surrounding them disappears, leaving them distinct against a clear background (fig. 16).

These different phases of nuclear organization are almost identical with those previously described for *Trichonympha campanula*. There is some suggestion in figure 16 of plate 13 that the chromosomes are arranged in pairs, as in *Trichonympha*. This point, with an exact count of the number of chromosomes, could not be as clearly made out here as in the other species, owing to the small number of individuals under observation. The number of chromosomes seems to be slightly less than that of *Trichonympha*, though this cannot be stated with certainty. In the prophase this seems to be forty-eight (pl. 13, fig. 15), with a reduction to twenty-four in the later stages (pl. 14, figs. 18–22). The process of pseudosynapsis by means of which this reduction takes place, cannot be figured from our material but is evidently similar to the same process in *Trichonympha*.

Metaphase: This stage also has been lacking in our material. The appearance of the following anaphase (pl. 14, fig. 17) would suggest in part the probable mode of procedure. In the late prophase the nucleus had taken its place close against the paradesmose (pl. 13, figs. 15, 16), and elongated until its length is equal to that of the paradesmose. Spindle fibers are formed from the ends of the paradesmose or bases of the centroblepharoplasts, and to these the chromosomes become attached, with a single fiber from each pole attached to the end of the chromosome lying nearest it. The nuclear membrane remains intact throughout the entire process of mitosis.

Anaphase: A slight shortening of the spindle fibers assists in the separation of the chromosomes, but apparently this is more dependent upon a lengthening of the entire nucleus in the equatorial plane than upon any other factor. The spindle fibers show but little contraction up to the time of their disappearance in the late telophase, and the chromosomes are not drawn to the poles (pl. 14, fig. 23). The nucleus becomes greatly elongated and constricted in the middle until the two halves are connected by a slender line of nuclear material. The paradesmose also increases in length.

As the chromosomes separate the heterochromosome may usually be found near the ends of the two groups (pl. 14, fig. 19). As in *Trichonympha* it is the lagging chromosome and is apparently the last one to divide. In a later stage this assumes a coiled shape preparatory to the formation of the vesicle by which it is later enclosed (fig. 20).

Its course in the prophase has not been followed but it apparently retains its isolated position throughout.

Telophase: As the two centroblepharoplasts with their related structures move towards opposite ends of the cell, the slender band connecting the two halves of the nucleus becomes ruptured, while the paradesmose fades out and begins to disappear at its middle portion (pl. 14, fig. 21). The daughter nuclei at this time are spindle-shaped both ends drawn out to a slender point, with the chromosome lying in a roughly subparallel band near the center. The point of the nucleus opposite the poles is withdrawn (fig. 20), the spindle fibers disappear, and the nucleus loses its connection with the centroblepharoplast (fig. 18) and begins to round up. This part of the process is usually completed before the chromosomes begin to undergo reorganization (pl. 14, figs. 22, 23, 25). Plasmotomy may occur before this takes place (fig. 25) or it may be delayed until reorganization of the nucleus and the reformation of the neuromotor system of each daughter cell has been completed (fig. 24).

RELATIONSHIPS

The close similarity of the various phases of division, combined with the striking resemblances in their morphologieal characters, indicate at once a close relationship between *Leidyopsis* and *Trichonympha*. These resemblances are found in the differentiations of the ectoplasm, the neuromotor system, and the interrelations of its various parts, the lack of a distinct cytostome, and the division of the endoplasm into a uniformly granular anterior portion and a posterior part filled with coarse alveoli and food particles, indicative of holozoic nutrition.

The differences between these two genera are few, but are important from a taxonomic standpoint. The most striking one is found in the number and arrangement of the flagella. In *Trichonympha* these cover two-thirds or more of the surface of the body and are divided into three distinct groups, an anterior, middle and posterior group. The anterior group is composed of long flagella, the middle or lateral group, which is the largest in extent, of short, cilia-like flagella, and the posterior group of flagella, which are twice or even three times the length of those in the anterior group. In *Leidyopsis* the middle and posterior groups of flagella are lacking, leaving only the group of long flagella at the anterior end of the body.

In view of the taxonomic importance attached to the number of flagella in other orders of the Flagellata, where these organelles serve as the chief features of classificatory value, these differences in their flagellar coating seem to create a generic distinction between the two forms and not a specific one only. We have therefore proposed a new genus in the order of Trichonymphidae for this form with the following characters:

Leidyopsis gen. nov.: Trichonymphidae with ectoplasmic differentiations found only on the anterior third of the body, the remainder of which is covered with a thin pellicle; neuromotor system consisting of one anterior group of long flagella, oblique fibers, longitudinal myonemes, basal granules and centroblepharoplast. One species only, the type, *Leidyopsis sphaerica* sp. nov. from intestine of *Termopsis angusticollis* Walker from Berkeley, California.

SUMMARY

1. *Leidyopsis sphaerica* is a flagellate of the *Trichonympha* type, with the same structural characteristics but with a lower type of evolutionary development.

2. It is characterized by the presence of a neuromotor system consisting of a highly developed centroblepharoplast, oblique fibers, basal granules and related flagella, restricted to a single anterior zone, a differentiated ectoplasm on the anterior third of the body, surface ridges from the crests of which spring the flagella, and an alveolar layer. Longitudinal myonemes are found in the endoplasm.

3. The nucleus is anterior in position and is distinguished by the presence of a heterochromosome contained within a small vesicle.

4. Nutrition is holozoic but its methods of feeding are unknown.

5. Division is of the trichonymphid type. The centroblepharoplast, with its connecting motor organelles and ectoplasmic structures, divides longitudinally with the formation of an extra-nuclear paradesmose between them as the two parts separate.

6. Division of the chromosomes is longitudinal, and takes place prior to the appearance of division in other structures of the body. About forty-eight chromosomes are formed. This number is reduced in pseudosynapsis to twenty-four.

7. The nuclear membrane remains intact throughout mitosis, with the spindle fibers arising from the ends of the paradesmose and centroblepharoplast and passing through it.

8. *Leidyopsis* is a member of the family Trichonymphidae Kent, in the order Hypermastigina Grassi. It stands close to the genus *Trichonympha*, to which it is related both morphologically and in its division processes.

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Berkeley, California.

LITERATURE CITED

Kofoid, C. A., and Swezy, O.

1919*a*. Parasites of the termites. I, On *Streblomastix strix*, a polymastigote flagellate, with a linear plasmodial phase. Univ. Calif. Publ. Zool., 20, 1–20, pls. 1, 2, 1 fig. in text.

1919*b*. Studies on the parasites of the termites. II, On *Trichomitus termitidis*, a polymastigote flagellate with a highly developed neuromotor system. *Ibid.*, 20, 21–40, pls. 3, 4, 2 figs. in text.

1919*c*. Studies on the parasites of the termites. III, On *Trichonympha campanula* sp. nov. *Ibid.*, 20, 41–98, pls. 5–12, 4 figs. in text.

EXPLANATION OF PLATES

All figures of *Leidyopsis sphaerica* gen. nov., sp. nov., from *Termopsis angusticollis* Walker, stained with iron haematoxylin and drawn with camera lucida. Magnification 800 unless otherwise stated.

PLATE 13

Fig. 1. Optical section of trophozoite showing thickness and extent of ectoplasm, centroblepharoplast and nucleus with the two portions of endoplasm. × 300.

Fig. 2. Vertical view of anterior end showing centroblepharoplast with central endoplasmic core, oblique fibers and nucleus slightly misplaced.

Fig. 3. Centroblepharoplast with a few of the radiating oblique fibers.

Fig. 4. Nucleus of the vegetative trophozoite; note the inner region filled with a coarse network with granules interspersed along its nodes, the surrounding alveolar zone and the outer granular region beneath the nuclear membrane.

Fig. 5. Nucleus showing the heterochromosome and its vesicle. Alveolar region has disappeared.

Fig. 6. Early prophase nucleus showing formation and splitting of the chromosomes.

Fig. 7. Early prophase with the alveolar region breaking up.

Fig. 8. Same stage as in figure 7.

Fig. 9. Another phase of the breaking up of the chromatin network.

Fig. 10. Anterior portion of the body, showing the cuplike depression with its covering operculum, the tip of the centroblepharoplast, and the surface ridges.

Fig. 11. Nucleus showing the full number of chromosomes.

Fig. 12. Beginning of the splitting of the centroblepharoplast and ectoplasmic structures.

Fig. 13. A slightly later stage of same process as shown in figure 12.

Fig. 14. Separation of the centroblepharoplast with the completion of the paradesmose connecting them.

Fig. 15. Same stage as in figure 14.

Fig. 16. Prophase of nucleus and the first appearance of the spindle fibers. Dark areas at the end of fibers are parts of the centroblepharoplasts.

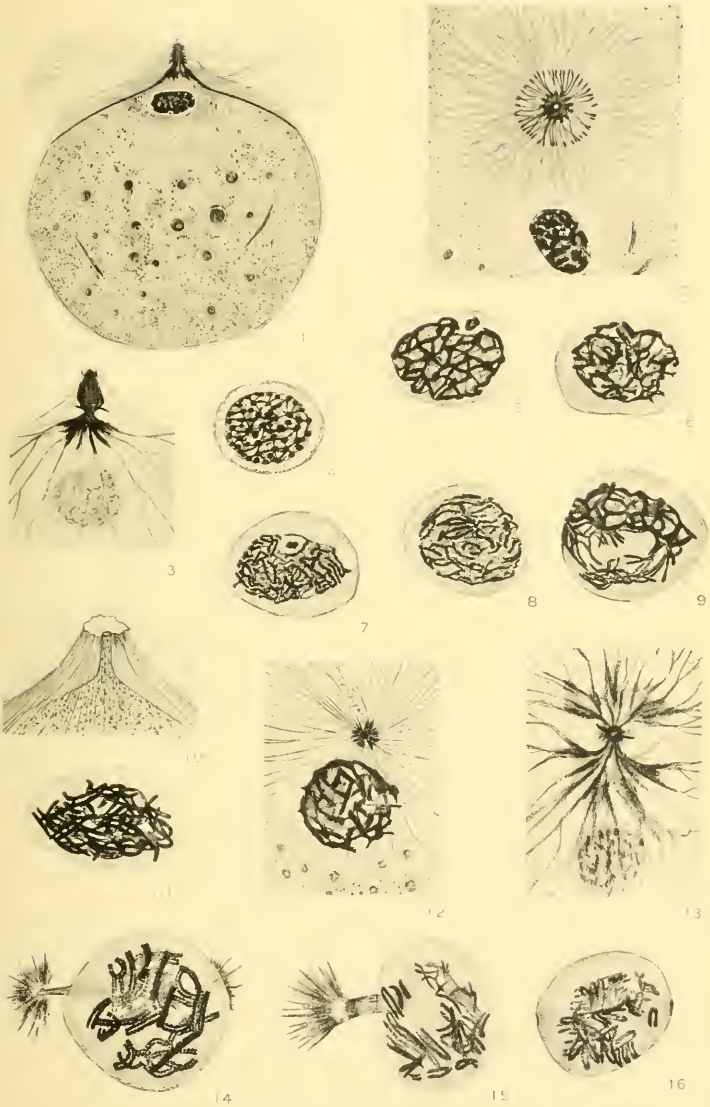

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PLATE 14

Fig. 17. Anaphase; note flagella and other ectoplasmic structures attached to the centroblepharoplasts. × 300.

Fig. 18. Telophase; paradesmose has been absorbed, the spindle fibers and connection of nucleus and centroblepharoplast are lost, but nuclear reorganization has not yet begun.

Fig. 19. Enlarged nucleus of figure 17. One lagging heterochromosome may be seen.

Fig. 20. Telophase of nucleus; spindle fibers still present as well as attachment to centroblepharoplast. Heterochromosome may be found near ends of the other chromosomes. × 1250.

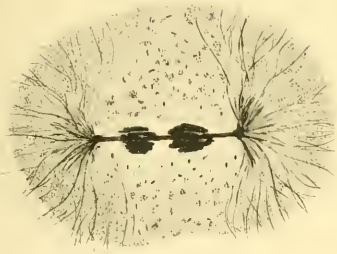
Fig. 21. Telophase; remains of paradesmose are seen above nuclei; flagella are omitted and the oblique fibers shown with their attachment to the centroblepharoplasts.

Fig. 22. Telophase of nucleus. It has rounded up and the chromosomes are losing their parallel position preparatory to forming a network.

Fig. 23. Telophase: beginning of constriction of the body. Note different relative positions of nuclei.

Fig. 24. Telophase; nuclear reorganization nearly complete as well as the formation of the new ectoplasmic structures. The alveolar zone, oblique fibers and flagella are shown.

Fig. 25. A daughter flagellate, after plastmotomy, which here has taken place before the reorganization of the nucleus.



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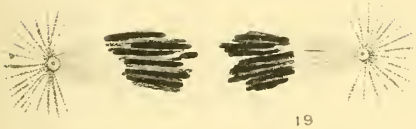

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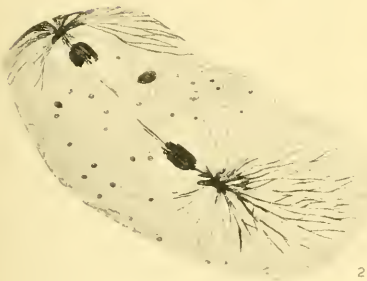

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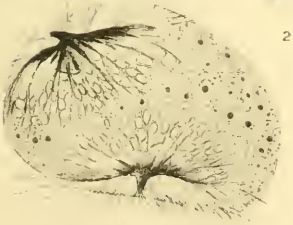

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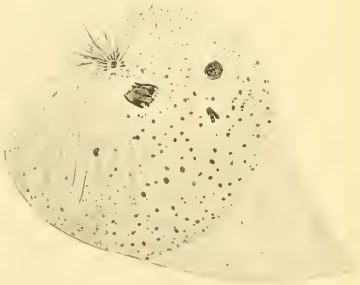

25

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Nos. 14 and 15 in one cover. April, 1918 ..... .15

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Index, pp. 537-545.

Vol. 18. 1. Mitosis in *Giardia microti*, by William C. Boeck. Pp. 1-26, plate 1. October, 1917 ..... .35

2. An Unusual Extension of the Distribution of the Shipworm in San Francisco Bay, California, by Albert L. Barrows. Pp. 27-43. December, 1917. .20

3. Description of Some New Species of *Polynoidae* from the Coast of California, by Christine Essenberg. Pp. 45-60, plates 2-3. October, 1917 ..... .20

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5. *Crithidia euryophthalmi*, sp. nov., from the Hemipteran Bug, *Euryophthalmus convivus* Stål, by Irene McCulloch. Pp. 75-88, 35 text figures. December, 1917 ..... .15

6. On the Orientation of *Erythropsis*, by Charles Atwood Kofoid and Olive Swezy. Pp. 89-102, 12 figures in text. December, 1917 ..... .15

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12. Differentials in Behavior of the Two Generations of *Salpa democratica* Relative to the Temperature of the Sea, by Ellis L. Michael. Pp. 239-298, plates 9-11, 1 figure in text. March, 1918 ..... .65

13. A Quantitative Analysis of the Molluscan Fauna of San Francisco Bay, by E. L. Packard. Pp. 299-336, plates 12-13, 6 figs. in text. April, 1918 ..... .40

14. The Neuromotor Apparatus of *Euplotes patella*, by Harry B. Yocom. Pp. 337-396, plates 14-16. September, 1918 ..... .70

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Nos. 17 and 18 in one cover. January, 1919..... .35
Index in preparation.

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2. The Pteropod *Desmopterus Pacificus* (sp. nov.), by Christine Essenberg. Pp. 85–88, 2 figures in text. May, 1919 ..... .05

3. Studies on *Giardia microti*, by William C. Boeck. Pp. 85-136, plate 1, 19 figures in text ..... .60

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2. Studies on the Parasites of the Termites II. On *Trichomitus Termitidis*, a Polymastigote Flagellate with a Highly Developed Neuromotor System, by Charles Atwood Kofoid and Olive Swezy. Pp. 21–40, plates 3–4, 2 figures in text. July, 1919 ..... .25

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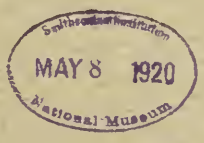
ON THE MORPHOLOGY AND MITOSIS OF CHILOMASTIX MESNILI (WENYON). A COMMON FLAGELLATE OF THE HUMAN INTESTINE

BY
CHARLES A. KOFOID AND OLIVE SWEZY



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CONTENTS

	PAGE
Introduction	117
Acknowledgments	119
Morphology	119
The active flagellate	119
The neuromotor system	123
The encysted flagellate	129
Mitosis	133
Discussion	135
Summary	137
Literature cited	138

INTRODUCTION

The present paper records certain observations on the free and encysted stages of *Chilomastix mesnili* (Wenyon), a common flagellate of the human digestive tract, made, in part during the course of examinations of stools of soldiers, primarily for *Endamoeba dysenteriae*, and in part at Berkeley in conjunction with the work of the Division of Parasitology of the Bureau of Communicable Diseases of the California State Board of Health. The army examinations were made at the United States Debarkation Hospital No. 3, New

York, N. Y., from December 28, 1918, to May 30, 1919, on 3301 stools from 2300 men who had been overseas, mainly in France, and from 576 men, chiefly of the medical detachments and food handlers, who had seen only home service. In the former group there were 97 persons infected or 4.2 per cent, and in the latter 20, or 3.5 per cent. At Berkeley 1836 stools of 534 persons have been examined, with 28 cases or 5.3 per cent of infection.

Although a common and widely distributed parasite of man, this organism has often been regarded as a tropical form. It has undoubtedly been confused, in much of the clinical literature, with other intestinal flagellates, especially with *Trichomonas*, and its structure, relationships, and encysted stages have been imperfectly described and figured and sometimes even misinterpreted as those of another flagellate. Conclusions as to its pathogenicity are also at variance.

The data here presented add to our knowledge of its wide distribution, and give, for the first time, a full analysis of its finer structure, especially of its neuromotor apparatus and of its mitosis in the encysted stage. This affords a satisfactory basis for conclusions as to its systematic relationships, a suggestion as to the holdfast function of the cytostome, and a critical basis for the accurate detection of this genus and its differentiation from the other parasites with which it is associated in the intestine of man and other mammals.

We present a full analysis of the structure of *Chilomastix*, especially of the neuromotor system, and by the homology of its elements find that *Chilomastix* is closely related to *Giardia*, and is a transition form from the monozoic to the diplozoic polymastigote flagellates.

Our analysis has added the following organs to those hitherto detected: the centrosome, nuclear rhizoplast, peristomal rhizoplast, peristomal fiber, paradesmose, and the spiral groove (as a permanent organ). We have also detected and analyzed intracystic mitosis. Our interpretations of the precise relations of the flagella to the blepharoplasts and of the organs of the cytostomal rim, which we designate as parastyle and parabasal, differ from those of previous writers and make possible a fuller comparison of *Chilomastix* and *Giardia*, and establish intimate relationships between the two genera.

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Acknowledgments are made to Lt. Col. E. H. Schorer, M.C., U.S.A., in charge of the Army Laboratory, for his efforts to provide facilities and staff for the work, and to 2nd Lt. S. I. Kornhauser for his technical aid in making preparations upon which this work is based.

A grant from the Division of Medicine and Related Sciences of the National Council of Research made possible the coöperation of the junior author.

MORPHOLOGY

The Active Flagellate

The *shape of the body of Chilomastix* is normally elongate pyriform, with the broad end anterior and the sides more or less convex, according to the degree of extension or contraction. Its length is generally two to four times its greatest width which is 0.2 to 0.3 of the total length from the anterior end. The anterior end is rather broadly rounded, sometimes flattened dorso-ventrally, and the curvature of the two sides is dissimilar. This dissimilarity is masked in the other irregularities due to the spiral groove. This asymetry is fundamental; it is caused by the relative positions of nucleus and cytostome, but is variously modified by the movements of the latter and by the torsion of the body. In ventral view (fig. A) the cytostome lies to the right side of the body (left of the figure) and the nucleus to the left. These relations persist in the cyst and at mitosis.

Posteriorly the body contracts rather abruptly into a short tapering tail, whose length is 0.2 to 0.3 of the total length. This tail may stretch out in a long thread, sometimes nearly as long as the body, when autotomy of the cytoplasm is in process, showing its great plasticity. It is apparent that the cytostome of *Chilomastix* is morphologically more highly differentiated than that of *Trichomonas, Eutrichomastix,* or *Embadomonas.* Specialization in function may well attend this structural development. Adhesion of the parasite to the intestinal wall of the host by *Giardia,* and possibly also by *Chilomastix,* is certainly one factor of no small clinical significance

in providing a structural and functional basis for the persistence of infections by these two flagellates during and after palliative treatment.

The *dimensions* of the active flagellate are quite variable, both in size and in extension. Twenty active individuals averaged 19.6μ in length, with a range of 13 to 24μ. Stained flagellates range from 9.6

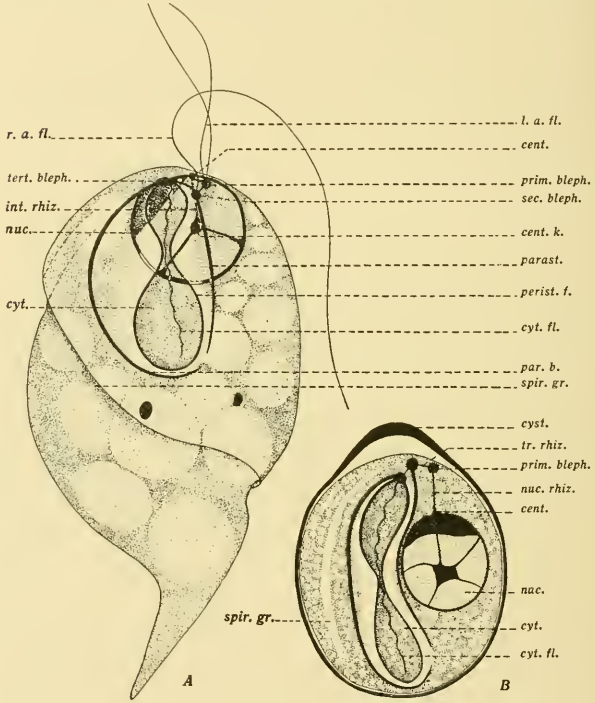

Fig. A. *Chilomastix mesnili* (Wenyon). Normal flagellate viewed from the ventral or oral side and showing all the structures of the body. × 6370.

Fig. B. Cyst of *Chilomastix mesnili*, viewed from the ventral or oral side. × 6370.

Abbreviations: *cent.*, centrosome; *cent. k.*, central karyosome; *cyst*, cyst wall; *cyt.*, cytostome; *cyt. fl.*, cytostomal flagellum or undulating membrane; *int. rhiz.*, intranuclear rhizoplast; *l. a. fl.*, left anterior flagella; *nuc.*, nucleus; *nuc. rhiz.*, nuclear rhizoplast; *par. b.*, parabasal body; *parast.*, parastyle; *perist. f.*, peristomal fiber; *prim. bleph.*, primary blepharoplast; *r. a. fl.*, right anterior flagellum; *sec. bleph.*, secondary blepharoplast; *spir. gr.*, spiral groove; *tert. bleph.*, tertiary blepharoplast; *tr. rhiz.*, transverse rhizoplast.

to 15μ, having contracted in the process of fixation. The small forms, 3μ in length, noted by Prowazek and Werner (1914), Wenyon (1915), and Chalmers and Pekkola (1918), may be *Chilomastix*. We find such small forms with the larger ones but have not been able to prove critically that they are *Chilomastix*.

The *cytoplasm* of *Chilomastix* is enclosed in a very resistant, elastic pellicle, which permits rapid changes in contour and deformation. It is crowded in the active stage with vacuoles of varying size, some of which contain bacteria (pl. 15, fig. 4), though generally they show only a fluid material. We find no evidence of a specialized region of ingestion in the cytostomal pouch, though the locality where the parastyle and parabasal are in juxtaposition is apparently adapted to this end. There is no specialized structure for the elimination of dejecta. This appears to be accomplished, in some instances, by the autotomy of cytoplasm and its contents at the posterior tip. In one instance a long rod-shaped bacillus was seen lying transversely in such a terminal mass.

The *spiral groove* (*spir. gr.*, figs. A, B) is a peripheral modification of the cytoplasm, seemingly peculiar to the genus *Chilomastix*, which is intimately connected with the very evident torsion of the body in the active flagellate stage. It is a special trough or channel impressed in the cytoplasm of the body, running from the dorsal face of the anterior end posteriorly in a spiral course from the mid-dorsal to the right side and obliquely across the ventral face to the left, making approximately a full turn in its sinistral course. This trough in contracted, stained individuals is a narrow, shallow channel about 0.25 of the diameter of the nucleus in width (pl. 15, fig. 1). In active flagellates it may be a deep, wide trough, which may be as wide as the nucleus anteriorly and cleave the body for nearly a third of its diameter, or it may be a narrow depression tapering posteriorly and terminating near the very tip. The floor of this groove is somewhat less coarsely granular than the rest of the peripheral cytoplasm. It is evidently a contractile area varying in the depth of its indentation. In the cyst (*spir. gr.*, fig. B; pl. 16, fig. 10) it is a meridional, faintly marked, clear streak on the right side of the nucleus and cytostome, running nearly from pole to pole. It appears to have lost entirely all trace of the torsion of the active flagellate stage after the encysted phase is established.

The *nucleus* (*nuc.*, fig. A) is a spheroidal, rarely ellipsoidal, structure located close to the anterior border of the body in the active

flagellate, where it usually is appressed against the blepharoplasts (pl. 15, fig. 5). In other cases it is slightly withdrawn, spinning out the rhizoplast between the centrosome on its anterior face and the primary blepharoplast. It lies to the left of the cytostome, asymmetrically and excentrically in the cytoplasm as a whole, both in the free and encysted stages, thus adding an important factor in the fundamental asymmetry of the organism.

As encystment approaches, and even before the cyst wall is formed, the nucleus withdraws from the anterior end posteriorly to a position slightly in advance of the middle of the cyst.

The close physical connection between the blepharoplasts at the base of the flagella and the nucleus, the metabolic center of the cell, throughout the period of flagellar activity, is indicative of the intimate relation which the nucleus bears to these energy-expending structures. It can not be merely the physical tug of the moving flagella which pulls the nucleus to its anterior position, for the blepharoplasts retain this anterior location when the nucleus moves posteriorly, the connection between them being retained merely by the slender nuclear rhizoplast. The rounding-up process in the cytoplasm of the encysting individual doubtless exerts some pressure leading to spatial readjustments, but the translation of the nucleus appears to be out of proportion to this single factor. In the active phase of the organism the nucleus is nearest to the center of metabolic activity, and in the passive phase of encystment it tends to assume a place as near as possible to the center of the cytoplasmic mass, the cytostomal pouch appearing to hold it off from the fully central location.

The presence of the centrosome on its anterior border gives evidence of a permanent polarization of the nucleus. The distribution of the chromatin within the nucleus lends support to this interpretation. There is a tendency for the chromatin, in the encysted stage, to accumulate at or near the anterior and the posterior poles of the nucleus, adacent to and opposite to the centrosome (pl. 16, figs. 8, 9), in plaques flattened against the membrane. In numbers of instances a radial fiber can be traced from the centrosome (fig. B) to the central karyosome, as in *Giardia*, where the intranuclear rhizoplast occupies a similar position. Several radial fibers within the nucleus are often found in the cysts. In other instances (pl. 16, figs. 11, 14) the chromatin is broken into several smaller blobs or plaques, which are distributed on the membrane irregularly and are sometimes connected by linin threads to the central karyosome. The intranuclear rhizoplast

is found less frequently in *Chilomastix* than in *Giardia* and is not so readily demonstrated. In some nuclei (fig. A; pl. 15, fig. 5) the chromatin is confined to a thin uniform sheet on the inner face of the nuclear membrane and to the central karyosome.

The *central karyosome* (fig. A, *cent. k.*) is a small, spheroidal or angular body at or near the center of the nucleus, joined to the centrosome by the intranuclear rhizoplast (*int. rhiz.*) and elsewhere to the peripheral chromatin masses by radial linin fibers.

The Neuromotor System

In *Trichomonas* the centroblepharoplast is the common point of origin of all fibrillar parts of the neuromotor system and generally lies close to the nuclear membrane. From it diverge the flagella, marginal fibril, paradesmose, rhizoplast, and, at mitosis, the paradesmose. In *Chilomastix* a more complex condition exists in that the centroblepharoplast complex is broken up into no less than four granules, each giving rise to two or more outgrowths, and all connected in sequence on a fiber which has its origin in the central karyosome of the nucleus.

We use the term neuromotor system here, as elsewhere (Kofoid, 1916; Kofoid and Swezy, 1919), to designate the integrated fibrillar system uniting the karyosome, centrosome, blepharoplasts, flagella, and other motor organs, and the fibers of the oral region into one continuous, structural unit. In *Chilomastix* this consists (fig. A) of the centrosome (*cent.*), three blepharoplasts (*prim. bleph., sec. bleph., tert. bleph.*), nuclear rhizoplast (*nuc. rhiz.*), transverse rhizoplast (*tr. rhiz.*), three anterior flagella, one posterior cytostomal flagellum (*cyt. fl.*), one parastyle (*parast.*), the parabasal body (*par. b.*), and the peristomal fiber (*perist. f.*). Taken together these constitute the locomotor and feeding organs of *Chilomastix*. It is not improbable that the cytostomal region has also a holdfast function.

This system is most clearly demonstrated in the encysted stage, in which the cytoplasm is free from confusing and obscuring food particles, and, what is far more important, the migration of the nucleus from the anterior end to its median-lateral position exposes the blepharoplasts and their connections. The approach of mitosis permits a wider separation of the elements and a clearer inspection of their structure and relations, especially of the blepharoplast complex, the parabasal, and parastyle. It is essential to have thoroughly

decolorized cysts and to study lateral as well as ventral views to obtain a clear conception of this organ system.

The *centrosome* (figs. A, B, *cent.*) in *Chilomastix* is a discrete and separate structure, independent, in both free and encysted stages, of the blepharoplasts. There is no centroblepharoplast here, as in *Trichomonas,* where the two organs are combined in one granule, but the relations of the two are similar to those found in *Giardia.* It is a small granule flattened against the anterior end of the nucleus where it forms a small hemisphere on the nuclear membrane. As mitosis ensues in the cyst, it divides and one daughter centrosome migrates posteriorly on the membrane of the nucleus (pl. 16, figs. 13, 15), spinning out the paradesmose between the two. In early stages the paradesmose is applied to the nuclear membrane (pl. 17, figs. 17, 19), but, in later stages, as the daughter nuclei part, it stretches out between them across the intervening cytoplasm (pl. 17, fig. 23). At no stage have we seen the centrosome detached from the nuclear membrane.

The *blepharoplast* in *Chilomastix* is a complex of three granules. In the free stages it is lodged on or near the anterior end of the nucleus and its structure is generally obscured by the crowding of organs anteriorly in a restricted area (pl. 15, figs. 2–6). It consists of three granules or individual blepharoplasts, which may be designated, according to their positions in relation to the nucleus, as the primary (figs. A, B, *prim. bleph.*), secondary (*sec. bleph.*), and tertiary (*tert. bleph.*) blepharoplasts respectively. In the free stage they are appressed against one another so that there may appear only two blepharoplasts, and the free flagella seem to emerge rather close together. Closer inspection, however, reveals the fact that the right flagellum is slightly removed from the other two. In stained flagellates (pl. 15, figs. 2–6) the nucleus crowds upon the blepharoplasts, obscuring the centrosome and rhizoplasts, while the two blepharoplasts of the right side generally appear as a single granule, often somewhat larger than the tertiary blepharoplast. The two (in reality three) granules usually lie at the anterior end of the nucleus and cytostome, but only one of them is actually attached to the peristomal fiber.

It is in the encysted stage that the structure, individuality, and relationships of the three blepharoplasts can be clearly distinguished (pl. 16, figs. 7, 8) as follows. The left or *primary blepharoplast* (fig. B, *prim. bleph.*) gives rise to two anterior flagella (pl. 15, fig. 1), is

connected posteriorly to the centrosome on the anterior face of the nuclear membrane by the nuclear rhizoplast (fig. B, *nuc. rhiz.*) and transversely to the secondary blepharoplast (fig. B, *sec. bleph*) by the transverse rhizoplast (fig. B, *tr. rhiz.*). It is somewhat widely separated from the other two blepharoplasts at the anterior end of the cytostome in this stage, and also from the nucleus which has migrated postero-laterally. Because of its connection with and more intimate relationship to the nucleus, this blepharoplast should be regarded as primary and the other two as of secondary and tertiary status.

The right anterior or *secondary blepharoplast* (fig. A, *sec. bleph.*) in the free flagellate (pl. 15, fig. 4) lies close to or appears to be fused with the right posterior or tertiary one in a more or less elongated body. In some cysts not heavily decolorized the two granules can not be differentiated. In some favorably oriented and strongly decolorized cysts it is possible to distinguish the secondary (*sec. bleph.*) from the tertiary blepharoplast (*tert. bleph.*) and to see the short peristomal rhizoplast connecting the two (pl. 16, fig. 10). The secondary blepharoplast gives rise anteriorly to the single right flagellum which is slightly detached from the other two (pl. 15, fig. 3), and posteriorly to the straight parastyle which underlies the left rim of the cytostome, and also to the short peristomal rhizoplast which joins this secondary blepharoplast to the tertiary blepharoplast.

The *tertiary blepharoplast* (fig. C, *ter. bleph.*) is at the anterior end of the rim of the cytostome. It gives rise anteriorly to the peristomal rhizoplast connecting it with the secondary blepharoplast and posteriorly to the undulating cytostomal flagellum, to the looped peristomal fiber (*perist. f.*) and to the curved parabasal (*par. b.*) which is attached to and posteriorly underlies the peristomal fiber. It is often obscured by the secondary blepharoplast and by the thickened anterior ends of the peristomal fiber.

The *peristomal fiber* is a fine thread encircling the so-called cytostome in its immediate rim and originating in the tertiary blepharoplast. Its proximal ends, as they emerge from this granule, are thickened and continue so for 0.25 to 0.33 of the length of the cytostome, while beyond this section it is a delicate fibril which encircles the remainder of the opening. Owing to its location in the immediate surface of the body, and to its overlying the parastyle on the left side and the parabasal on the right, it is easily confused with these structures or overlooked, especially if the stain is not sufficiently decolorized. Furthermore, the varying degrees of contrac-

tion and expansion of the cytostome give differing pictures of the relations of the peristomal fiber to the underlying organs.

The deeply stained periphery of the cytostome of *Chilomastix* owes its appearance to two structures whose analysis in preparations is most difficult and whose morphological interpretation offers an interesting problem. These organs are the parastyle and the parabasal body. They so much exeeed the peristomal fiber in volume and stainability that they appear largely to have been the structures interpreted as the cytostome and drawn as its margin by previous investigators (Brumpt, 1913; Prowazek and Werner, 1914; Kuczynski, 1914; and De Fonseca, 1916), except Chalmers and Pekkola (1918). The last named note the distinction between the peristomal fiber and the underlying stained structures.

The *parabasal* (fig. A, *par. b.*) is a deeply staining, curved rod which runs posteriorly from the tertiary blepharoplast. Its course follows approximately that of the right margin of the cytostome but posteriorly it extends beyond this opening and lies deep in the cytoplasm, curving to the left around the posterior end of the oral poueh, extending anteriorly for a short distanee on its left side, and ending in a fine point near the posterior end of the parastyle.

In some cases (pl. 16, fig. 8) it appears to adhere to the peristomal fiber for a short distance, in others it is free from that fiber throughout its whole length. The parabasal is imbedded in the right wall of the oral pouch. In imperfectly decolorized individuals this whole wall may retain the stain or the fiber be buried in the lateral sheet of stained material. The curvature of the parabasal varies according to the state of its contraction. When the cytostome is wide open the parabasal may be exposed (pl. 16, fig. 10) inside of the cytostomal opening, and when the lips are contracted and the cytostome reduced to a constricted, slipper-shaped opening (pl. 16, fig. 11), the parabasal is outside of the opening on the right side of the body. It usually entends posteriorly beyond the end of the cytostomal opening (pl. 16, fig. 13) but may be included within it (pl. 17, fig. 16). These varying pictures of its relations to the cytostome are records of the mobility and exhibit stages of the contraetion of the overlying peristomal fiber. As Chalmers and Pekkola (1918) state, the inner and outer lips may contract so as almost to touch each other, forming overhanging lobes (pl. 16, fig. 8) in the anterior half of the opening. Such a lobe may form on one side only. These movements of the cytostome are suggestive of the food-gripping function observed by the authors cited

above and also of a sucker or holdfast action of this region which we have occasionaly seen in the free flagellates. The structure, lateral position, and relation to the cytostome of this parabasal suggest its analogy to the antero-laterally located, deeply stained, curved structure near the rim of the cytostome of *Giardia*, which we have called the anterior peristome (Kofoid and Christiansen, 1915), but we believe it to be the homologue of the parabasal, and that the peristomal fibers of the two genera are homologues. It appears to bear the same morphological relation to the cytostomal flagellum that the parabasal bears to the undulating membrane in *Trichomonas*. Chalmers and Pekkola (1918) also have called this structure the parabasal.

We have found no evidence that the parabasal of flagellates is a motor or contractile organ and have elsewhere (Kofoid, 1916) advanced the interpretation that it is a reserve body connected with metabolism consequent upon the motor functions of the neuromotor system. While its changes in shape in *Chilomastix* are far more noticeable than those of the parastyle, this might well be due to the intense activity of the adjacent cytostomic flagellum and of the peristomal fiber and parastyle, rather than to its own intrinsic movement.

In one point our findings are at variance with those of Chalmers and Pekkola (1918), namely, the origin of the parabasal. They state (p. 225) that it arises from the second or anterior chromatic particle, our secondary blepharoplast. We find that in every case where it is possible to separate the anterior and posterior right blepharoplasts the parabasal is joined to the *posterior* one only, that is to the right posterior or tertiary blepharoplast.

The *parastyle* (fig. A, *parast.*) is a slender, straight, or slightly curved rod, much smaller and usually more decolorized than the parabasal. It originates from the secondary blepharoplast, and runs posteriorly in the left wall of the oral pouch, almost to the posterior end of that cavity, sometimes overlapping the recurved end of the parabasal and more or less obscured by the peristomal fiber above it.

The relative shapes of parastyle and parabasal, as seen in a series of free flagellates and cysts, are instructive as to their relative functions. While the parabasal exhibits a great variety of shapes showing the contractility and mobility of the region in which it lies, the parastyle is straighter, has much slighter curvature, and its shapes likewise appear to be correlated with the state of contraction of the cytostome, as though it were passively moved by the activity of that region. There is lack of evidence that it is an active motor organ. It appears

to have the same relation to the neuromotor system that the right axostyle has in the neuromotor system of the right side of *Giardia*. We have called it a parastyle because it is alongside the cytostome. It differs from the axostyle of *Giardia* in not ending in a free flagellum. The parallelism in the persistence in the cysts of *Giardia* of axostyles and parabasals and of the parastyle and parabasal in *Chilomastix* is significant of the homology of these structures in the two genera.

The *flagella* of *Chilomastix* are four in number and fall into three categories, the two left anterior (fig. A, *l. a. fl.*), the right anterior (*r. a. fl.*) and the cytostomal (*cyt. fl.*). The three anterior ones are free throughout their whole course, while the fourth is attached to the cytoplasm and proceeds posteriorly within the cytostome as an undulating membrane. The two left anterior flagella arise from the primary blepharoplast. In locomotion these two flagella act in unison, beating together in the backward stroke, the wave of contraction in the inner one slightly preceding that in the outer one. These two flagella are thrown down against the left side of the body while the right anterior one beats in the opposite direction and is thrown down against the right side of the body with a tendency to lie for a time in a trailing position. It arises from the secondary blepharoplast. The length of these three anterior flagella is about equal to the length of the body.

The *cytostomal flagellum* (fig. B, *cyt. fl.*), or undulating membrane, originates from the tertiary blepharoplast and runs posteriorly in the floor of the oral pouch within the cytoplasm to or near to the end of the cytostome. In action it moves in rapid waves of contraction running posteriorly and almost equalling the width of the oral pouch in amplitude. The rate, in moribund individuals whose quiescence permits observation, is two to four beats per second. The amplitude of the lateral movement dies out distally. We have never been able to see any evidence of a free end of this flagellum. The fiber which constitutes it persists in the encysted stage and can be traced by careful focussing.

This flagellum corresponds to the filament in the margin of the undulating membrane of *Trichomonas, Trichomitus,* and *Eutrichomastix,* the cytostomal flagellum of *Chilomastix* being, in reality, a diminutive undulating membrane enclosed in the cytostomal pouch. It lies adjacent to the parabasal the location and length of which are correlated with those of this flagellum, as in the trichomonad flagellates. Its presence has led to confusion of *Chilomastix* with *Trichomonas* in many clinical accounts of human flagellates.

The Encysted Flagellate

The cysts (fig. B; pls. 16, 17) of *Chilomastix* are small, ovoidal, pyriform, or lemon-shaped bodies in some cases remarkably similar in general appearance to the smaller cysts of the species of amoeba with which they are associated in the intestine of man. In fresh smears in physiological salt solution the cysts are bluish green bodies with distinct walls and often a few highly refractive granules strewn within. In iodine-eosin stain the cysts quickly become tinged with the iodine to a light yellow, and, according to the amount and diffusion of the contained glycogen, gradually assume an olive or brownish tint. If the glycogen is massed, the brown color is very evident in the glycogen vacuole, if it is diffuse, a dark olive or brownish tint spreads through the whole cyst. If glycogen is not present the cyst remains yellow. As the iodine penetrates, the outlines of the nuclear membrane and of the fibrillar sling in the walls of the cytostome become visible. The cysts are not uniformly lemon-shaped. In not a few cases all trace of the pyriform contraction is lacking and the cyst is ovoidal, spheroidal, or broadly ellipsoidal (pl. 16, figs. 11, 12).

In the pyriform cysts the length is from 1.21 to 1.24 times the greatest diameter, which is slightly below the level of the middle of the body, assuming that the narrower end is the anterior one. They are thus broadly pyriform in contour. In other instances the neck of the cyst is not contracted and the contour becomes broadly ovoidal with the length 1.24 to 1.31 times the greatest diameter which is located at 0.6 of the total length from the anterior end. In still other instances the cyst may be spheroidal (pl. 16, fig. 8), with the longer axis reduced to 1.08 times the greatest diameter and the anterior projection forming only a very obscure and slight eminence. It is only in lateral view that this departure from sphericity is detected.

The cyst varies from 6.5 to 9μ in length and averages 7 to 7.5μ, while its greatest diameter is from 5.8 to 7.5μ and averages 6.5μ. Its dimensions are thus very close to those of a red blood corpuscle, and to the small races of *Endolimax buetschlii* and *Endamoeba dysenteriae*, an important point in the microscopical diagnosis of these infections.

The *cyst wall* is a transparent, homogenous substance, without structural modification except at the tip of the stout projection, where it is locally thickened across the end to double the thickness elsewhere,

or to about 0.25μ. This localized thickening, together with the projection, forms convenient diagnostic characters for the identification of these cysts, and for their distinction from ovoidal and ellipsoidal cysts of *Endolimax buetschlii* or from the occasional ellipsoidal or subspheroidal one of *Endamoeba dysenteriae*.

The body of the flagellate may not completely fill the cyst, retracting away from the anterior projection in stained cysts. Elsewhere it lies in intimate contact with the wall of the cyst.

The *orientation of the cyst* is determined by the position of the flagellate within it. Normally or usually the anterior end of the flagellate, which is distinguished by the location of the compound blepharoplast, appears to be directed towards the tapering end of the cyst (pl. 16, figs. 7, 10). There are, however, cysts in which this relation of cyst and flagellate is reversed (pl. 16, figs. 9, 11). This condition appears to be brought about by the turning of the flagellate within the cyst after the wall is completed and the form and orientation of the cyst determined. The rounded subspheroidal cysts (pl. 16, fig. 8) may be caused by the rotations of the flagellate within the cyst during its more plastic condition.

The organs of the free-moving flagellate, with the exception of the free flagella, are all visible in the stained cysts. In stained cysts with diffuse glycogen (pl. 16, fig. 10) the cytoplasm was rather uniformly and finely alveolar. In a few cases the glycogen was clumped in clouds, rather diffusely margined, scattered in the cytoplasm (pl. 16, fig. 8). In stained cysts these glycogen masses are dissolved out, leaving coarser vacuoles (pl. 16, fig. 9). Very rarely was the glycogen condensed in one or more dense, deeply staining, iodophylic masses which became dark brown in iodine-eosin and dissolved out, leaving large vacuoles (pl. 16, fig. 7), when treated with aqueous solution, as in the iron haematoxylin staining method. Dobell and Jepps (1917) have figured two such glycogen-laden cysts.

The amount of glycogen within the cyst decreases with age, as it does in the glycogen-bearing cysts of the three species of human intestinal amoebae. There is great variation in the cysts in different stools in the amount of glycogen contained therein, but less variation among the cysts of a given stool. Older binucleate cysts lack the glycogen vacuoles.

The other cytoplasmic contents of the cyst are the chromatoidal bodies. These may or may not be present and when found vary greatly in number and location. They are ellipsoidal, ovoidal, or spheroidal

masses of deeply staining substance, taking, in iron haematoxylin, the same stain as the chromatin within the nucleus (pl. 16, fig. 11). These bodies are from 0.25 to 0.9μ in greatest diameter, homogenous in structure, and lie near the borders of the cytostome and nucleus. In these particulars they resemble the chromatoidal structures found near the parabasal in the cytoplasm of *Trichomonas* and called by us (1915) "chromidia." Their variability corresponds to that of the chromatoidal rods of the cysts of *Endamoeba*, with which they are associated in the faeces, and suggests that they are of the same nature, and meet similar needs of the organism during encystment. They contain reserve materials of unknown, but probably nuclear origin, contingent upon encystment.

The organelles of the flagellate are retained to an unusual degree in the encysted stage (fig. B). The nucleus (*nuc.*) is spherical, or spheroidal with its longer axis parallel to the major axis of the cyst. It is generally located a little in front of the middle of the body and to the right side of the cytostome, considerably posterior to its customary position in the free stage. It is 2 to 2.5μ in diameter. Its membrane may be very delicate with one, or several scattered chromatin plaques, or it may be rather heavily encrusted with chromatin in addition to one or two large plaques which appear to bear a fairly constant relation to the rhizoplast and axis of the body. These two plaques are usually unequal in size, the larger often anterior and at the junction of the rhizoplast with the nucleus, and the smaller one at or near the opposite pole. These relations are not constant, however, the posterior mass being the larger in some instances (pl. 16, fig. 8), while in others there is only a single anterior plaque (pl. 16, fig. 10). There is in most nuclei a central karyosome, less well-defined than in the free stage, and often somewhat excentric in location. From it there pass peripherally fine threads to the chromatin plaques or other smaller chromatin aggregates on the nuclear membrane. A clear, fluid-filled zone lies between the central karyosome and the peripheral chromatin.

The structure of the nucleus is suggestive of a permanently polarized organization not unlike that found in *Giardia* by Kofoid and Christiansen (1915) and by Boeck (1917). This polarization is further emphasized by the presence of a small granule on the anterior end of the nucleus into which the rhizoplast passes. We interpret this granule as the centrosome (*cent.*, fig. B), since its location and morphologieal relations to the nucleus, rhizoplast, and blepharoplast are

homologous with those of a like structure in *Giardia*, where this granule plays the rôle of the centrosome at mitosis. This centrosome is seen with difficulty, if at all, owing to its juxtaposition to the large chromatin plaque and sometimes to the position of the nucleus which may be so turned as to obscure it. At mitosis in *Chilomastix* this granule divides and the daughter centrosomes form the paradesmose between them as a fiber on the nuclear membrane (pl. 16, fig. 15).

A comparison of the position, structure, and relations of the blepharoplasts in the free flagellate with those of the encysted stage indicates that the nucleus in its postero-lateral migration to near the center of the cytoplasmic mass has not only drawn out the long rhizoplast from the left blepharoplast but has also parted the blepharoplasts more or less widely (pl. 16, figs. 8, 11).

In the encysted stage the primary blepharoplast (fig. B, *prim. bleph.*), has lost its two anterior flagella, is often quite small, dislocated laterally to the left and connected by the nuclear rhizoplast (*nuc. rhiz.*) to the centrosome (*cent.*) on the anterior face of the nucleus. The transverse rhizoplast (*tr. rhiz.*) joins it to the secondary blepharoplast (*sec. bleph.*) which has lost its single right flagellum. This granule and the preceding are often about equal in size to the primary blepharoplast. The tertiary blepharoplast lies near the ventral surface at the anterior end of the cytostome, receives the peristomal rhizoplast, and gives rise posteriorly to three structures: (1) to the cytostomal flagellum which, unlike the free flagella, persists as a deeply staining fiber during the early phases of the cyst, (2) to the curved parabasal, a deeply staining rod in the right wall of the oral pouch, and (3) to the peristomal fiber in the margin of the opening into the cytostome. The latter is so delicate and so superficial in location as to require great care in differentiating it from the underlying parastyle and parabasal.

The unity, fibrillar continuity, and elasticity of the neuromotor system is again demonstrated in the structure and behavior of the centroblepharoplast complex of *Chilomastix* during encystment. Though divided into centrosome and three blepharoplasts the fibrillar continuity of the complex is maintained unbroken in encystment and mitosis.

The cytostomal complex is clearly recognizable in the cyst, both in the iodine-eosin stain and in the iron haematoxylin, as an elongated or slipper-shaped loop with a contracted region anteriorly. A dark thread, the peristomal fiber, outlines the opening into the shallow

cavity of the oral pouch. The same structural peculiarities which exist in the neuromotor apparatus in the free flagellate are present in the cyst except for the free flagella. There is little if any change in the size of its constituent elements in the cyst.

The spiral groove of the body (fig. B, *spir. gr.*) loses its spiral course and becomes a faint meridional shadow at the right of the cytostome, distinguishable in the iron haematoxylin preparation as a narrow, clear region extending from the anterior end of the encysted flagellate nearly to its posterior end (pl. 16, figs. 10–14).

MITOSIS

Binary fission in the free flagellates has not been found in our stained faecal smears, though constant watch has been maintained for it. In the encysted stages, however, we have been more fortunate in that we have been able to secure a fairly representative series showing the main steps in the mitosis of this form in the first two divisions in the cyst. Many of the minute details of this process have thus far eluded us, as well as the plasmotomy of the two or more daughter flagellates thus formed and their liberation from the cyst.

The onset of mitosis is apparently not foreshadowed in the behavior of the chromatin contents of the nucleus, since this retains its usual structure until the new neuromotor organelles are at least partially developed (pl. 16, figs. 10, 11, 13, 14). The process is begun by the division of the centrosome and blepharoplasts. The earliest stages we have been able to find (pl. 16, fig. 13) show these granules duplicated and each set of blepharoplasts connected by the normal complement of rhizoplasts to the as yet undivided nucleus. The origin of the rhizoplasts appears to be by division of the original rhizoplasts. The centrosomes spin out between them as they separate a darkly staining thread, the paradesmose (pl. 16, figs. 13, 14), which lies on the nuclear membrane.

The duplication of other parts of the neuromotor apparatus occurs by outgrowth and not by division, as seems to be the case with the blepharoplasts and rhizoplasts. The new cytostome, and its peristomal fiber and associated parabasal, parastyle, and cytostomal flagellum, arise as outgrowths of the new secondary and tertiary blepharoplasts, appearing at first as minute loops and threads (pl. 16, fig. 13) which gradually enlarge until, in the final stages, they equal

the old organelles in size (pl. 17, fig. 24). The old cytostome, with its peristomal fiber, parabasal, parastyle, and cytostomal flagellum, remains intact throughout the process of mitosis, though later stages would seem to indicate that the entire complex is somewhat reduced in size and its cytostomal flagellum can no longer be found (pl. 17, figs. 22, 23). It retains its original position while the new cytostome, with its related organelles, follows the migrating centrosome and eventually comes to lie at the opposite end of the body (pl. 17, figs. 18–22). With the completion of mitosis and the disappearance of the paradesmose, the activity of the encysted flagellate somatella may bring the second neuromotor complex and nucleus back to their original anterior position, so that the two groups of organelles come to lie side by side (pl. 17, fig. 24). The mobility which we have observed in the dividing schizonts in binary and multiple fission in free trichomonad flagellates (see Kofoid and Swezy, 1915) appears to occur, to some extent, in the schizonts of *Chilomastix* while still within the cyst.

During the development of the new neuromotor organelles the daughter centrosomes move farther apart on the nuclear membrane (pl. 16, figs. 13–15; pl. 17, figs. 16–17) until they come to lie at opposite poles of the nucleus (pl. 17, fig. 18), drawing out between them the slender thread which forms the paradesmose. This may be seen in stained preparations as a dark line which follows the contour of the nucleus but may not be attached to it (pl. 17, fig. 20). In later stages, it extends through the cytoplasm between the centrosomes on the nuclear membrane of the two daughter nuclei (pl. 17, fig. 23).

The nuclear membrane is retained intact throughout the entire process of mitosis. It loses its encrusted chromatin before the formation of the spindle, becomes considerably enlarged and is drawn out into a spindle shape as mitosis progresses, and is finally parted by equatorial constriction into the two daughter nuclei.

The behavior of the chromatin and its formation into chromosomes we have not been able to follow in all details. The amount of chromatin apparently increases considerably with the onset of mitotic phenomena and decreases in the final phases, as may be noted by a comparison of figures 17 to 20 on plate 17 with the other figures given on the same and preceding plates. A spireme is formed as a rather thick, continuous thread (pl. 17, fig. 17) coiled in the axis of the nucleus. This spireme later shows transverse constriction into five unequal masses prior to its movement into the equatorial plate (pl. 17,

fig. 19). In the equatorial plate stage (pl. 17, fig. 18) the chromosomes appear as large, dense rounded masses, connected with the centrosomes at the poles by numerous spindle fibers. The number of these granules or chromosomes is rather difficult to determine at this stage, but in the anaphase (fig. 20) five were clearly distinguishable. They vary considerably in size, and stain intensely with iron haematoxylin. The spindle fibers disappear as the chromosomes move to the poles (pl. 17, fig. 22) and each granule becomes elongated in a narrow cone at its point of attachment to the pole of the nucleus where the centrosome lies.

In the reorganization of the nuclei the chromatin is found massed in groups of rather large granules at the center of the nucleus (pl. 17, fig. 24). Later the chromatin takes on the form of a small compact karyosome connected with the periphery by narrow fibers and the remainder of the chromatin collects on the nuclear membrane (pl. 17, fig. 23).

The plane of division of *Chilomastix* is morphologically longitudinal. This is shown in the prophase in the splitting of the blepharoplasts and their separation (pl. 16, figs. 13–15; pl. 17, figs. 16, 17). With the formation of the relatively large spindle in the rigid confines of the cyst some adaptation is necessary for the accommodation of the two schizonts. This adaptation appears in the initial posterior migration of one of the centrosomes carrying with it the newly formed neuromotor apparatus, resulting in an apparently transverse division of the nucleus (pl. 17, figs. 19–22).

It is probable that one or two other nuclear divisions follow in the cysts, though we have not been able as yet to find them in infected stools in which we have secured the first division by keeping the stools for as much as four days at room temperatures.

DISCUSSION

Chilomastix is asymmetrical in the distribution and function of the anterior flagella, in the location and organization of the neuromotor apparatus, and in the course of the spiral groove. This groove has the same torsion as the transverse flagellum and girdle of the dinoflagellates and the undulating membrane of *Trichomonas*. This torsion is sinistral. It has the same twist as the thread of a left-handed screw.

When binary fission occurs in the free and encysted stages the newly formed neuromotor apparatus, which is attached to the centrosome at one of the poles of the dividing nucleus and goes into one of the daughter individuals, has the same asymmetry as that of the parent individual which goes into the other daughter. The structures at the two poles are not mirror images each of the other. Bilateral symmetry of the two as yet unseparated daughter cells is not established by mitosis.

The structures which we have discovered in the neuromotor organization of *Chilomastix* are, in detail, similar to and homologous with those of the right cell in the binucleate Hexamitidae and most closely with those of the cell on the right side of *Giardia*. The centrosome, nuclear rhizoplast, blepharoplast complex, peristomal fiber, parabasal, and parastyle are homologous with the like organs in *Giardia*, save that the parastyle is represented by the axostyle with its free terminal flagellum in *Giardia*, but has no flagellum in *Chilomastix*. The three anterior flagella of the former are represented by the lateral, posterolateral and ventral flagella of the latter, while the undulating flagellum of the cytostome seems to have disappeared in *Giardia*. The asymmetry of the cytostomal region of the two genera is of like pattern and the spatial relations of this region to nucleus, centrosome and blepharoplast are strikingly similar in the two organisms.

It is obvious from the comparative morphology of these two genera that they are rather closely related and that the Hexamitidae, including *Giardia*, must be regarded as an offshoot from *Chilomastix* or its near relations. *Giardia* differs from *Chilomastix* in two very important particulars: (1) it is binucleate and (2) it is bilaterally symmetrical with the left cell the mirror image of the right, which is the morphological equivalent of *Chilomastix*.

This binucleate bilateral organism could be derived from sinistral *Chilomastix* only by a complete morphological reversal of the fundamental asymmetry of the left cell, with its nucleus and neuromotor apparatus, in the binucleate body and the coincident suspension of plasmotomy. Such a process, which is not unlike molecular reversal in principle, might establish the bilateral binucleate Hexamitidae.

The morphological relations of *Chilomastix* and *Giardia* are profoundly significant and instructive as to the method by which bilaterality has appeared among the highly specialized, asymmetrical, unicellular organisms.

SUMMARY

1. *Chilomastix mesnili* has a deep spiral groove running posteriorly from right over to left as a permanent cell organ distinct from but adjacent to the cytostome. It persists in the cyst as a meridional structure.

2. The neuromotor apparatus consists of centrosome, nuclear rhizoplast, three blepharoplasts and connecting rhizoplasts, the primary giving rise to two flagella, the secondary to one and to the parastyle, the tertiary to the parabasal, the peristomal fibril, and the cytostomal flagellum or undulating membrane.

3. The centroblepharoplast complex is thus subdivided into four granules, the centrosome and three blepharoplasts having continuous rhizoplast connections with the central karyosome of the nucleus.

4. The nucleus is polarized with the centrosome anterior and the spireme forms in its longitudinal axis. Binary fission in the cyst is morphologically longitudinal.

5. The blepharoplast-rhizoplast chain splits lengthwise at mitosis and the remainder of the neuromotor complex appears to be produced *de novo* by outgrowths from the blepharoplasts prior to the spireme stage. The daughter centrosomes are connected by a paradesmose.

6. In mitosis the nuclear membrane remains intact and its construction is spatially transverse. The daughter nuclei are for a time connected by the paradesmose but lie at opposite poles of the cyst, but may later change their position.

7. The neuromotor system of *Chilomastix* is strikingly similar to that of the right half of *Giardia* in symmetry and its constituent elements.

8. The two daughter individuals have the same symmetry; they are each equivalent to the right half of *Giardia*.

9. The bilateral symmetry of the two-celled *Giardia* could arise only by a complete morphological reversal from the sinistral to the dextral type of one of the two daughter schizonts at mitosis.

10. The genus *Chilomastix* is closely related in structure to and may be the source of the bilateral binucleate Hexamitidae.

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LITERATURE CITED

Boeck, W. C.
1917. Mitosis in *Giardia microti*. Univ. Calif. Publ. Zool., **18**, 1–26, pl. 1, 1 fig. in text.

Brumpt, E.
1913. Précis de parasitologie. Ed. 3 (Paris, Masson), xxviii + 1011 + 32 pp., 4 pls., 698 figs. in text.

Chalmers, A. J., and Pekkola, W.
1918. *Chilomastix mesnili* (Wenyon, 1910). Ann. Trop. Med. and Parasit., **11**, 213–264, pls. 6–7.

Dobell, C., and Jepps, M. W.
1917. On the three common intestinal entamoebae of man, and their differential diagnosis. Brit. Med. Journ., 607–612, 14 figs in text.

Fonseca, O. O. R. da
1916. Estudos sobre os flagelados parasitos dos mamiferos do Brazil. Mem. Inst. Oswaldo Cruz, **8**, 5–40, pls. 1–2, 4 figs. in text.

Kofoid, C. A.
1916. Biological and medical significance of the intestinal flagellates. Proc. Sec. Pan Amer. Sci. Cong., 1915–1916, **10**, 546–565.

Kofoid, C. A., and Christiansen, E. B.
1915. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–54, pls. 5–8, 1 fig. in text.

Kofoid, C. A., and Swezy, O.
1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad. Arts and Sci., Boston, **51**, 289–378, pls. 1–8, 7 figs. in text.
1919. Studies on the parasites of the termites. I–IV. Univ. Calif. Publ. Zool., **20**, 1–116, pls. 1–12, 9 figs. in text.

Kuczynski, M.
1914. Untersuchungen an Trichomonaden. Arch. Prot., **33**, 119–204, pls. 11–16, 4 figs. in text.

Prowazek, S., and Werner, H.
1914. Zur Kentniss der sog. Flagellaten. Arch. Schiffs-u. Tropenhyg., **18**, 155–167, pl. 10, 1 fig. in text.

Swellengrebel, N. H.
1917. Ueber die Cystenbildung des *Chilomastix mesnili* Wenyon. Arch. Prot., **38**, 89–93, pls. 1, 2, 1 fig. in text.

Wenyon, C. M.
1910. A new flagellate (*Macrostoma mesnili* n. sp.) from the human intestine, with some remarks on the supposed cysts of *Trichomonas*. Parasit., **3**, 210–216, pl. 16, 2 figs. in text.

EXPLANATION OF PLATES

All figures of *Chilomastix mesnili* were drawn with camera from Schaudinn-iron haematoxylin preparations at a uniform magnification of 4800. Finer structures have been worked out with high-power monobjective binocular microscope with strong illumination, fluorite and apochromatic objectives, wide-angle condenser and immersion oil between slide and condenser.

PLATE 15

Fig. 1. Normal trophozoite showing the spiral groove, the cytostome with its peristomal fiber, cytostomal flagellum, parabasal and parastyle, the nucleus anteriorly located, and the three blepharoplasts and centrosome.

Fig. 2. Small flagellate which is preparing to encyst or may have just escaped from a cyst.

Fig. 3. Free flagellate showing the deep notch of the spiral groove. The blepharoplast complex appears as a single granule.

Fig. 4. The large vacuoles show the position of glycogen masses in the living flagellates. The black rods represent ingested bacteria. Spiral groove obliterated by contraction.

Fig. 5. *Chilomastix* viewed from the "dorsal" or aboral side. The cytostome is partly engulfed by the deep spiral groove.

Fig. 6. *Chilomastix* of the more slender type.

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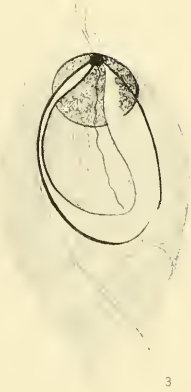

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PLATE 16

Fig. 7. Encysted *Chilomastix* showing the typical pyriform cyst with its wall at the anterior end thickened.

Fig. 8. Spheroidal cyst with large chromatoidal bodies near the parabasal and parastyle.

Fig. 9. Cyst viewed from the aboral side. The body within the cyst has become inverted with the anterior end at the large end of the cyst.

Fig. 10. Cyst showing normal appearance of encysted flagellate. Spiral groove shown as clear line on the right side. Parabasal exposed within the cytostome.

Fig. 11. Ovoidal cyst containing numerous chromatoidal bodies in cytoplasm. Body inverted within the cyst.

Fig. 12. Subspheroidal cyst.

Fig. 13. Prophase of mitosis. Centrosome, blepharoplasts and rhizoplasts have divided, and the new cytostome with parabasal and parastyle has appeared.

Fig. 14. Prophase of mitosis. A slightly later stage with the cytostome enlarging.

Fig. 15. A still later stage of the prophase, with the new cytostome fully developed and the chromatin of the nucleus beginning its reorganization for mitosis.

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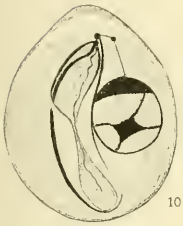
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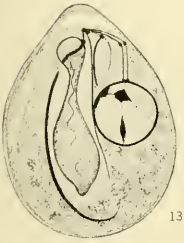
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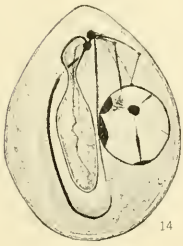
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PLATE 17

Fig. 16. Prophase of mitosis. The backward migration of one centrosome and the new cytostome has begun.

Fig. 17. Prophase of mitosis. The chromatin has become arranged in a coiled spireme in the axis of the nucleus.

Fig. 18. Equatorial plate stage. Note the posterior position of the new cytostome and the paradesmose connecting the two centrosomes. Body inverted within the cyst.

Fig. 19. Abnormal type of anaphase.

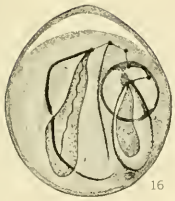
Fig. 20. Anaphase. Five chromosomes of unequal size may be distinguished. Note the separation of the paradesmose and the nuclear membrane.

Fig. 21. Anaphase. Note the spindle fibers still connecting the two groups of chromosomes. Body inverted in cyst.

Fig. 22. Late anaphase. Note the conical shape of the chromosomes. The old cytostome appears reduced in size.

Fig. 23. Telophase. Reorganization of the nuclei completed but paradesmose still persisting. Note shortening and thickening of the parabasals and decrease in the paradesmose.

Fig. 24. Reorganization of the neuromotor apparatus of each set completed but that of the nuclei still in progress. Note absence of paradesmose, anterior location of the blepharoplasts, and parallel position of the schizonts.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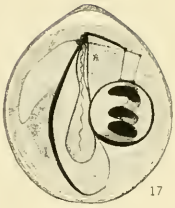
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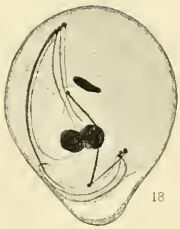
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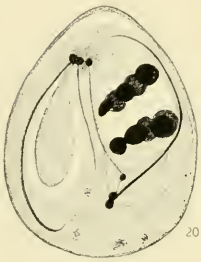
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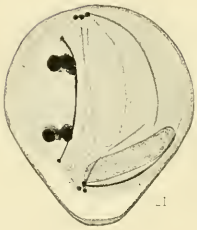
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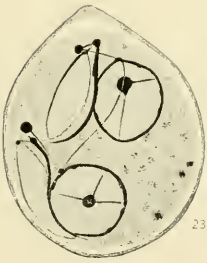
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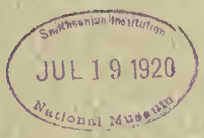
BY
CHARLES ATWOOD KOFOID



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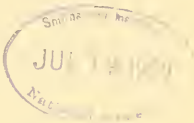


CONTENTS

	PAGE
Introduction	145
Discussion	147
Cercomonas not as yet proved to be a human parasite	147
Chilomastix davainei (Moquin-Tandon)	151
Trichomonas hominis (Davaine)	155
Trichomonas of the mouth and respiratory tract	156
Tetratrichomonas, a synonym of Trichomonas	159
Tritrichomonas gen. nov. for trichomonads with three flagella	160
Giardia enterica (Grassi)	160
Summary	164
Literature cited	165

INTRODUCTION

The conquest and control of the intestinal protozoan infections of man, which investigation is showing to be both frequent and varied, involves among many other important factors the accurate determination and differentiation of the various flagellate and other protistan species of the intestinal fauna. This is fundamental for the detection of etiological factors of disease, especially in view of the prevalence of varied and multiple infections.

For purposes of comparative protozoological investigation and of clinical and therapeutic progress, it is eminently desirable that the

nomenclature of these organisms be established upon as sound a basis as may be feasible with our present knowledge of the organisms and in the light of the accumulated literature, much of which is confusing, incomplete, and even contradictory.

The early microscopists who first explored the invisible living world turned their crude instruments to the dejecta of man and found therein, as elsewhere in organic material in the early stages of decay, a horde of bacteria and of flagellates feeding thereon. They were hampered in their investigations by the rapid movements of the monads, by the fact that the technique of modern staining was undeveloped, by the poor definition of their lenses which failed to reveal the number and relation of the flagella, by the lack of organization of scientific bibliography, and by the absence of monographic works. The science of protozoology was, as yet, undeveloped.

These observers saw the commoner flagellates of man, sketched and described them briefly and imperfectly, especially as to the flagella which now constitute the bases of classification. They often misinterpreted the undulating membrane as a row of cilia, and either failed entirely to see the other flagella, or did not correctly resolve their numbers. Added to these difficulties is the fact that clinicians and zoologists have each alike been uninformed, in many cases, as to the literature in the other's field when they have ventured to christen some newly discovered flagellate from the intestine of man.

As a result of these conditions the nomenclature of these flagellates has been in a continual state of confusion from the early discoveries of Müller (1773), Donné (1837), Davaine (1854, 1860, Lambl (1859, 1875), and Grassi (1879, 1881) to the present time.

After some experience at the U. S. Army Laboratory, New York City, and in the Division of Parasitology, Bureau of Communicable Diseases, California State Board of Health, of the practical difficulties in the identification of intestinal flagellates with existing facilities, and a persistent effort to establish on sound bases the names at present widely but not universally used for these organisms, and after a careful perusal of the pertinent literature, the following conclusions are offered as a solution of the correct nomenclature, under the Zoological Code of Nomenclature, of the common flagellates of man. We offer our conclusions for criticism, with the hope that they may be recognized as conforming to the Code of Nomenclature, and that they may conduce ultimately to stability of nomenclature in this common ground of medicine and zoology.

DISCUSSION

Cercomonas Not as Yet Proved to be a Human Parasite

This genus was originally described by Dujardin (1841) for free-living flagellates from infusions. It was characterized by the possession of one anterior flagellum and a posterior prolongation. It was therefore natural that human parasitic flagellates in which there was seemingly one anterior flagellum and a caudal process should be referred to this genus. In the clinical literature to the present day, this genus is a convenient catchall for most inadequately determined flagellates of man. This reference results in part from the fact that the first flagellate to be described from the intestine of man was called *Cercomonas* by Davaine (1854).

The genus *Cercomonas,* however, has not been critically established as a parasite of man by any of the many extensive surveys of the protozoan intestinal fauna which have been made in recent years in connection with the World War. No competent protozoologist has described, figured, and critically discussed any *Cercomonas* as an *undoubted* parasite of man in connection with these tens of thousands of stool examinations. There are, however, many reports of occurrences of *Cercomonas* and of *Cercomonas hominis*, and some of *C. parva* and *C. crassicauda*, but they all fall, in my opinion, into some one of the following categories.

1. Uncritical allocations to *Cercomonas* of some flagellate not assignable to *Trichomonas* or *Giardia*. In most cases, if not all, the organism present and thus reported was *Chilomastix davainei* Moquin-Tandon, a name which we adopt in lieu of *C. mesnili* (Wenyon). This conclusion rests upon statistical evidence such as that of Matthews and Smith (1919) who compile the results of 23,024 stool examinations of 4068 persons, mainly dysenterics, with 669 infections of *Giardia intestinalis,* or 16.4 per cent, 148 of *Chilomastix mesnili,* or 3.6 per cent, and 29 of *Trichomonas intestinalis,* or 0.7 per cent; and that of Kofoid (1920), who compiles the results of 3218 examinations of 2876 soldiers, with 168 *Giardia intestinalis,* or 5.9 per cent, 117 *Chilomastix mesnili* or 4.1 per cent, 6 *Trichomonas intestinalis* or 0.2 per cent, and 4 occurrences of *Embadomonas intestinalis*, and 8 of *Tricercomonas intestinalis.* It thus appears that, according to statistical probabilities, any flagellate in addition to

Giardia and *Trichomonas* present in any numbers in any group of human cases, is *Chilomastix*.

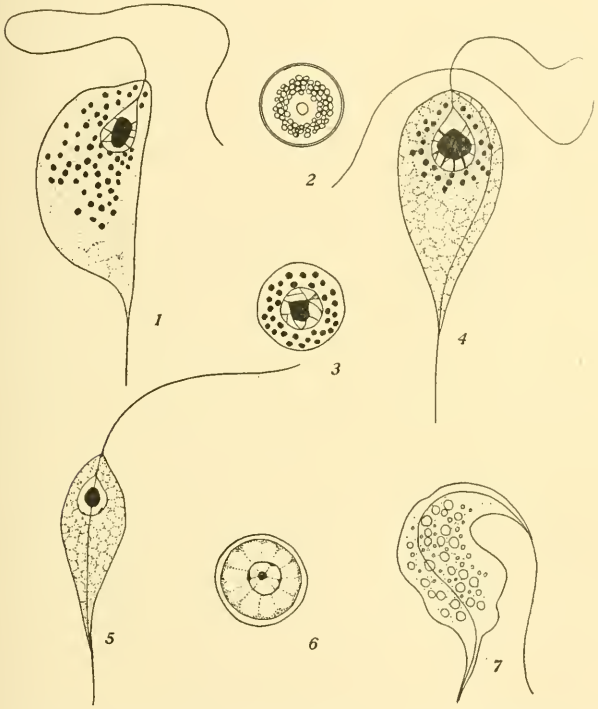
Additional support of this conjecture is derived from the fact that nearly all of the clinical writers reporting *Cercomonas*, such for example as Guastalla (1909), Mello Leitao (1913), Prentiss (1915), Chace and Tasker (1917), and Simon (1918), have taken no note of *Chilomastix*. The same is true of certain reports of larger numbers of cases surveyed, as for example those of Christie (1915), 3 per cent in 100 natives of Sarawak, and Smithies (1918), who reports 36 cases in 1000 examinations in Augustana Hospital, Chicago. It is very probable that some of the earlier clinical literature reviewed by Janowski (1897) reporting *Cercomonas* deals in reality with *Chilomastix*.

2. In the second category belong certain cases of critical determination of flagellates in stool cultures or in stools as species of *Cercomonas*, such as that of Wenyon (1910*a*) who found *Cercomonas longicauda* in a culture from a human stool, and that of Hartmann and Chagas (1910), who found *C. parva* n. sp. in a like culture. The first of these species occurs in infusions, but neither was regarded by the authors as of parasitic origin in the cultures. These investigators give critical figures of the organisms and there is no doubt that they were correctly refered to the genus *Cercomonas*.

Less certainty as to status attends the reports of the occurrence of *Cercomonas hominis* in the stools of dysenteric patients by Fantham and Porter (1916*a*) and of Fantham (1916), who also later (1916*b*) reported two infections of *C. parva* in 1305 dysenteric patients in England, and 3 in 14 special cases in hospitals in Liverpool. No figures or accounts of the organisms thus identified are given by these authors and the two reports appear to deal with the same group of examinations. Porter (1918) found 10 infections by *C. parva* among 375 South African natives, but none in 104 autopsies, but in her account details are lacking which would establish the parasitism of the organisms. Woodcock (1916) reports one case of *Cercomonas* among 26 dysenteric patients at the Dardenelles. Castellani (1917) lists it from the Balkans, and Sangiorgi (1919) from Italian soldiers at Venice. Cutler and Williamson (1919) list *C. crassicauda* among the intestinal flagellates of man without citation of its occurrence as such. Since *Chilomastix* was well known to these investigators and they are familiar with intestinal flagellates and the literature thereof, the organisms they have referred to *Cercomonas* must belong to one of two genera, either to *Cercomonas* or to *Craigia*. If to the former, they

may have been dealing with the coprophilic infections of stale stools; if the latter, with active flagellates of parasitic origin belonging to *Craigia* rather than *Cercomonas*.

3. Judging from the occurrence of *Cercomonas* in culture from human stools, as shown by Wenyon (1910) and Hartmann and Chagas (1910), it is probable that this genus contains coprophilic species which



Figs 1–4. *Cercomonas longicauda* Dujardin. After Wenyon, 1910, figs. 9, 10, 19. Magnification about 2000. Fig. 1. Lateral view of free stage showing flattened side along which the posteriorly directed intracytoplasmic flagellum runs. Figs. 2 and 3. Encysted stages, cyst wall not shown.

Figs. 5–7. *Cercomonas parva* Hartmann and Chagas (1910, pl. 4, figs. 4, 2, and 12). × 2500. From culture of human faeces. Fig. 5. Free stage, showing axostyle with terminal posterior flagellum. Fig. 6. Free stage from life. Fig. 7. Encysted stage with cyst wall.

multiply in the human stool after its evacuation from the bowel, and that the stool may be inoculated either after evacuation by cysts from dust or water, or by living flagellates in hospital receptacles, or by cysts which have safely passed through the intestine but have not developed at body temperatures. It may well be that the reports of *C. hominis* by Fantham and Porter (1916) and of *C. parva* by the former, and by Porter (1918), and by other competent protozoologists, rest upon the coprophilic basis, rather than upon a parasitic one. There has been as yet, however, no adequate evidence presented to establish the presence of either the living *Cercomonas* or of their cysts in fresh stools in numbers characteristic of parasitic infections. While it may be that *Cercomonas* is represented by a parasitic species in the human intestine, a survey of the literature shows that the evidence thereof is quite unconvincing.

4. It is possible that the uniflagellate phase of *Craigia hominis* (Craig) which does occur in fresh human stools (Craig, 1911, and Barlow, 1915) might be mistaken in fresh preparations for *Cercomonas*. *Craigia* usually lacks the tapering posterior end of the species of *Cercomonas*, but may sometimes be attenuate posteriorly. In stained preparations it shows an unusually large central karyosome which is not characteristic of *Cercomonas*. It is likewise accompanied by the amoeboid stage and sometimes by the multiple encysted phase in the human stool. The possibility of the confusion of *Craigia* with *Cercomonas* in much of the literature should be borne in mind.

From a consideration of the points above enumerated we conclude that there is no proof that *Cercomonas* is represented among the human intestinal flagellates, though it may occur in the human coprozoic fauna. The species thus found are *Cercomonas longicauda* Dujardin and *C. parva* Hartmann and Chagas. The species *Cercomonas hominis* Davaine is not today recognizable as a member of the genus *Cercomonas* in human stools. Davaine's (1860) forms belong to other genera, as we shall show later.

It is highly desirable that all flagellates reported as *Cercomonas* by any investigator of human flagellates or disease associated therewith, be carefully worked out from stained preparations and accurately figured, that cultures be attempted, and that necessary precautions be taken to determine whether the flagellate has a coprozoic or a parasitic source.

Chilomastix davainei (Moquin-Tandon)

The question now arises as to what flagellates Davaine (1854, 1860) really had as the basis of his description of *Cercomonas hominis* vars. *A* and *B*. After considerable experience in the examination of living human intestinal flagellates from fresh stools and cultures thereof (except *Giardia*), and a critical reading of Davaine's earlier (1854) and later (1860) accounts of the organisms discovered by him, we conclude that (1) Davaine had two different flagellates, his vars. *A* and *B*. This rests on his own decision, the accumulated statistical evidence of recent years, and the contrasts in his descriptions and figures; (2) the larger form, his var. *A*, is *Chilomastix*, and the smaller one, *Trichomonas*. This decision is supported by the relative sizes of *Chilomastix*, 19.6 (13–24)μ and *Trichomonas*, 5–15μ. *Chilomastix* sometimes has the dimensions as small as those given by Davaine. A second and most conclusive evidence is Davaine's (1860, p. vi, not in 1854) statement "trait longitudinal vers l'extrémité anterieure, donnant l'apparence d'un orifice buccal?;". *Chilomastix* is the only human flagellate with well-defined, elongated cytostome except *Embadomonas*, which is much smaller and apparently very rare. This cytostome serves today as the best available criterion for the certain identification of *Chilomastix* in both free and encysted stages. The cytostome of *Trichomonas* is not elongated nor so bordered by a cytostomal rim as to be easily seen as is that of *Chilomastix*. The tapering posterior end (extrémité amincie) is also a distinguishing characteristic. Davaine's (1860) figures of his form may be harmonized without difficulty, save in the matter of the number of flagella, with our present knowledge of *Chilomastix*.

We therefore conclude that Davaine's (1860) *Cercomonas hominis* var. *A* is the organism later described by Wenyon (1910) as *Macrostoma mesnili* and now generally called *Chilomastix mesnili*. A survey of the literature indicates that Brumpt (1912) and Chalmers and Pekkola (1917), investigators of *Chilomastix*, *all agree that Cercomonas hominis* var. *A* Davaine is the same organism as *Chilomastix mesnili*, though Brumpt later (1913) places an interrogation mark after the synonym and Chalmers and Pekkola quote *Cercomonas hominis* as a synonym *pro parte* without noting his varieties.

Since Davaine's *Cercomonas hominis* is a complex of two species, the withdrawal of either one of these under a new name leaves *hominis* as the residual name for the species not withdrawn. This withdrawal

was first made by Moquin-Tandon (1860, p. 398) who called Davaine's larger form (his var. *A*) *C. davainei*, and the smaller one (his var. *B*) *C. obliqua* (p. 399). Accepting page sequence in Moquin-Tandon (1860) as establishing the priority of *C. davainei* as the first withdrawn species, var. *B* becomes residual and retains the name *hominis*, while *C. obliqua* Moquin-Tandon becomes a synonym of *C. hominis*. Davaine's sequence (of vars. *A* and *B*) cannot be used to establish the priority of the subsequent reviser's (Moquin-Tandon) names. It follows from the evidence here presented that the correct name for the human intestinal flagellate which Davaine called *Cercomonas hominis* var. *A* is *Chilomastix davainei* (Moquin-Tandon).

There remains one obscure feature of this problem, namely the question of priority between Davaine's (1860) "Traité des Entozoaires" and Moquin-Tandon's (1860) "Éléments de Zoologie Médicale." Both bear the same date, were printed at the same press and were issued by the same prominent publishing house, J. B. Baillière et Fils, publishers for the Imperial Academy of Medicine in Paris. The English translation of Moquin-Tandon's work by R. T. Hulme apeared in London in the following year. The date of the preface in Moquin-Tandon's smaller book is September 1, 1859, and the flagellates are treated in the closing pages. The date of the preface of Davaine's larger book is September 30, 1859, but the flagellates are treated in the first pages of the book in a "Synopsis" paginated separately in Roman numbers.

The Bibliotheque Nationale of Paris in reply to an inquiry writes that "L'ouvrage de Moquin-Tandon *Elements de zoologie medicale* est daté de 1859 et figure au journal de la libraire (Dèpot légal) du 24 Septembre, 1859, sous le No. 8501. Le *Traite des Entozoaires* de Davaine figure du même journal du 3 Décembre, 1859, sous le No. 10774. Sauf retard l'editeur à effectuer le dèpot, il est dont un peu posterieur."

These dates evidently refer to registration merely, rather than to actual deposit of the volumes following publication, since the publisher dated them both 1860.

There is fortunately decisive internal textual evidence that Moquin-Tandon had before his eyes Davaine's figures when he wrote his descriptions of *Cercomonas davainei* and *C. obliqua*. We reproduce both Davaine's and Moquin-Tandon's descriptions herewith:

From Davaine's (1854) original article in which no specific names and no figures are given we quote:

"Les cercomonas des selles, au contraire, ont des organes qui ne laissent aucun doute sur leur nature animale; ils ont 8 millièmes de millimètre de longuer; leur forme est ovoïde, un peu variable; le tégument est mou, blanchâtre; un corpuscule ou deux, très-petit, nucléaire, peut quelquefois être perçu à l'intérieur. L'extrémité antérieure est pourvue d'un filament vibratile d'une minceur extrême, très-long, que l'on devine d'abord par l'agitation qu'il exerce dans le liquide ambiant, et que l'on n'aperçoit qu'à de courts intervalles et avec une attention soutenue; à l'extrémité opposée existe un autre filament plus épais, aussi long ou plus long que le corps, qui s'agglutine quelquefois aux corpuscules environnants et autour duquel, dans ce cas, la cercomonas oscille comme la lentille du pendula autour de sa tige. Ces cercomonas sont extrêmement agiles, ce qui rend l'observation de leurs caractères très-difficile. La cercomonas des selles du malade atteint de fièvre typhoïde m'a paru différer de celle des cholériques, en ce que le filament caudal s'insère un peu sur le côté du corps, et en ce qu'il offrait, en outre, un mouvement d'ondulation dans tout de contour."

From Davaine, "Traité des Entozoaires," 1860, p. vi:

CERCOMONAS DE L'HOMME (CERCOM. HOMINIS, DAVAINE)

Variété ou espèce *A*

Corps pyriforme, variable, long de 0 mm, 01 à 0 mm, 012; extrémité amincie se terminant par un filament caudal épais aussi long que le corps; filament flagelliforme antérieur situé à l'extrémité obtuse, opposé au précédent, très long (deux fois aussi long que le corps?) et mince, toujours agité, très difficile à voir; trait longitudinal vers l'extrémité antérieure, donnant l'apparence d'un orifice buccal?; point de nucléus bien appréciable. Locomotion assez rapide, quelquefois suspendue par l'agglutination du filament caudal aux corps environnants; l'animal oscille alors, comme un pendule, autour du filament.

Ces protozoaires existent en nombre quelquefois considérable dans les garderobes récentes des malades atteints du choléra.

Variété ou espèce *B*

Plus petite que la précédente: corps moins pyriforme, à contours moins arrondis, long de 0 mm, 008; deux filaments, l'un antérieur, l'autre caudal, situés un peu latéralement; longueur des filaments non déterminée; locomotion très rapide. Cette variété se rapproche des *Amphimonas*.

Protozoaires observés en grand nombre dans les déjections d'un individu atteint de fièvre typhoïde.

From Moquin-Tandon, "Éléments de Zoologie Médicale," pp. 398, 399:

"2° CERCOMONADE (Cercomonas).—M. Davaine a découvert, en 1853 dans les déjections encore chaudes des cholériques, une espèce de Cercomonade qui s'y trouvait en nombre très considérable.

Plus tard le même savant a observé deux fois le même animalcule dans les selles de quelques malades atteints de diarrhée simple. L'épidémie du choléra régnait encore.

J'ai désigné cet Infusoire, dans mes cours, sous le nom de Cercomonade de Davaine (*Cercomonas Davainei*). [Name in footnote.]

Ces animalcules présentent 8 millimètres de longueur. Leur corps est obové ou pyriforme, nn peu variable, très obtus en avant et fortement atténué. Leur

tégument est mou et blanchâtre. Un ou deux corpuscules, très petits, nucléaires, peuvent quelquefois être aperçus à l'intérieur. En avant, on remarque un filament vibratile d'une minceur extrême, très long et très flexueux, naissant brusquement de bord antérieur, que l'on devine d'abord par l'agitation qu'il exerce dans le liquide ambiant, et que l'on n'aperçoit qu'à de courts intervalles et aves une attention soutenue; à l'extrémité opposée existe un autre filament plus épais, du moins à son origine, se confondant avec le partie postérieure, de la longueur du corps ou un peu plus long, presque droit, roide, s'agglutinant quelquerois aux corpuscules environnants, et autour duquel, dans ce cas, la Cercomonade oscille comme la lentille du pendule autour de sa tige. . . .

Une antre espèce de Cercomonade a été rencontrée une fois chez un jeune homme atteint d'une fièvre typhoïde bien caractérisée, sans aucun phénomène cholérique. C'est encore à M. Davaine que nous devous sa connaissance.

Cette seconde espèce diffère un peu de la première; elle est plus petite et plus ovoïde; son filament antérieur paraît de même long et flexueux; il se détache moins brusquement. Le filament caudal naît un peu sur le côte; il est proportionnellement plus petit et ne se confond pas à son origine avec l'extrémité du corps. Cette Cercomonade offre un mouvement d'ondulation dans tout le contour. qui semble quelquefois légerèment sinueux.

On ponrrait appeler cette espèce Cercomonade oblique (*Cercomonas obliqua*)." [Name in footnote.]

8 9

Fig. 8. *Cercomonas hominis* var. *A*. After Davaine (1860, fig. 1 [1]). × 467. Note the undulating membrane characteristic of *Trichomonas hominis*.

Fig. 9. *Cercomonas hominis* var. *B*. After Davaine (1860, fig. 1 [2]). × 467. Note the cytostome characteristic of *Chilomastix davainei*.

In his earlier account Davaine (1854) makes no mention whatsoever of the smaller size and more ovoid form of his var. *B* which Moquin-Tandon notes. The latter must therefore have had Davaine's later figures and possibly also the text before him when he wrote his description. Davaine's figures appear only in his later (1860) work.

There is some question as to Moquin-Tandon's access to Davaine's text for he omits reference to the "orifice buccal" in *C. davainei* (*C. hominis* var. *A*) and states of *C. obliqua* (*C. hominis* var. *B*) "son filament antérieur paraît de même long et flexueux" while Davaine says of it "longeur des filaments non déterminée."

Moquin-Tandon was professor of medical natural history in the Faculté de Médecin at Paris and his book appeared in French and in English editions, but in the face of this publicity his treatment of Davaine's flagellates appears to have escaped the notice of all contemporary and subsequent parasitologists, including that of his com-

patriots Railliet (1889, 1895), Verdun (1907, 1913), Neveu-Lemaire (1908), and Brumpt (1910, 1913), as well as that of Leuckart and his followers. His names fulfill all requirements under the Code of Nomenclature and have a just claim for consideration.

In view of the internal evidence that Moquin-Tandon had before him Davaine's figures and his acknowledgment, "C'est encore à M. Davaine que nous devons sa connaissance" we award priority to Davaine's *Cercomonas hominis* over *C. obliqua* Moquin-Tandon, but establish his *Cercomonas davainei* on the reviser's privilege of naming one (but not both) of the two species included under Davaine's *C. hominis*.

Trichomonas hominis (Davaine)

As stated above, the human intestinal *Trichomonas*, the smaller species described by Davaine as *Cercomonas hominis* var. *B*, is the *Trichomonas* of the human intestine. The grounds upon which this conclusion rests are as follows.

(1) The statistical frequency in which *Giardia*, *Chilomastix*, and *Trichomonas* are revealed in any series of examinations of diarrheic stools, makes it highly probable that Davaine found *Trichomonas* in the course of his examinations of choleraic stools.

(2) There is no evidence that he had found *Giardia* except the phrase (1854, 1860) "un corpuscle, ou deux, très petit, nucléaire" in his account of his var. *A* (*Chilomastix davainei*). The two nuclei might have been due either to mitosis or to a confusion with *Giardia*. There is no additional evidence of *Giardia* whatever. There is nothing in his account of his var. *B* to suggest *Giardia*. We therefore eliminate this genus and have only *Trichomonas* left as a probability.

(3) Conclusive evidence that Davaine had found *Trichomonas* appears in his first paper (1854) where two characteristics are noted in the phrases "le filament caudal s'insere un peu sur le côte du corps et en ce qu'il offrait, en outre, un mouvement d'ondulation dans tout le contour." These are the undulating membrane and the postero-lateral departures of the free end of the lateral marginal flagellum. He omits reference to the undulations in his later (1860) treatise. They are the main features upon which identification is made today of the living *Trichonomas*. They are not together characteristic of any other human intestinal flagellate. *Chilomastix* has a cytostomal undulating membrane found only with greatest difficulty, and the posterior end

of the body is fairly symmetrical. *Tricercomonas* has a laterally displaced caudal prolongation but no undulating membrane. Davaine's *Cercomonas hominis* var. *B*, which has both characters, was therefore the human intestinal *Trichomonas* and its correct specific name is *hominis* on the ground that Davaine had two species in his original *C. hominis* and one of these, his var. *A*, was detached by Moquin-Tandon (1860) as *Cercomonas davainei*, leaving Davaine's var. *B* as the residual species to retain the specific name *hominis* on the principle of elimination. This name therefore replaces *T. intestinalis* Leuckart (1879) and *T. confusa* Stiles (1902) proposed later as names for the human intestinal *Trichomonas*.

Trichomonas of the Human Mouth and Respiratory Tract

There is much uncertainty as to whether or not there is more than one species of *Trichomonas* in man. Species have been described from the intestine (*hominis, intestinalis*), from the urogenital tract (*vaginalis*), from the mouth (*Tetratrichomonas buccalis, hominis*), and from the lungs (*pulmonalis*). Two points of view and two possibilities are open. (1) There is but one species in man which is modified by the tissues and secretions which surround it, thus giving rise to the morphological differences that have been detected among *Trichomonas* from the different organs. This is the view of Jollos (1913). (2) There are at least two species, the intestinal one and the one in the vagina. There is also the possibility that there is a third species in the mouth and that this may be the same as the one in the lung, though infection in the lung by the intestinal form carried there in the blood stream, or by invasion from hepatic abscess accompanying *Endamoeba dysenteriae*, is also possible. Culture and inoculation of *Trichomonas* from these different organs and much more morphological work accompanying these experimental investigations will be necessary to determine the number of species. For the present it seems advisable to treat the *Trichomonas* from the intestine (*T. hominis* (Davaine)), from the vagina (*T. vaginalis* Donné), from the mouth (*T. buccalis* (Goodey *and Wellings*)) and questionably that from the lung (*T. pulmonalis*), as separate species pending investigation.

The recent work of Bensen (1909), Brumpt (1913) and Lynch (1915*a*, *b*) is indicative that there are morphological distinctions between *T. hominis* and *T. vaginalis*, and the investigations of Goodey and Wellings (1917) upon the flagellate of the mouth is suggestive

that this may be a distinct species also; but more data are desirable on this matter.

The discoverer of the flagellate of the mouth was O. F. Müller, the early Danish explorer of the microfauna of ponds and ditches, who ransacked a culture made from the tartar between dirty human teeth and found therein (1773, 1776) and figured (1786) tiny organisms which he included in his genus *Cercaria* as *C. tenax*, together with dinoflagellates and larval stages of trematodes. These parasites from the mouth have not the slightest relation to larval trematodes, though their possession of a short tail afforded a justification for this allocation in the days of medieval morphology and systematics. His diminutive sketches show no flagella but the pyriform shape and the short asymmetrically curved, pointed, posterior projection are suggestive of the projecting axostyle and flagellum of *Trichomonas*.

Bory de Saint Vincent (1824) in one of the last volumes of the "Encyclopédie Méthodique" (*fide* Moquin-Tandon, 1860, p. 400) established the genus *Virgulina* for tailed animalcules and included therein Müller's species *V. pirenula*.

In 1838–1845 Mandl (*fide* Moquin-Tandon, 1860) rediscovered Müller's animalcules in the tartar about human teeth subjected to microscopical examination in distilled and boiled water. He records their dimensions as from 0.05 to several hundredths of a millimeter, possibly a typographical error for 0.005, and notes their lively movements. They were said to contain in their bodies calcareous elements which contribute to the deposition of tartar of the teeth.

Moquin-Tandon (1860) in his "Éléments de Zoologie Médicale" revives Müller's name *tenax*, follows Mandl in referring it to Bory's genus *Virgulina* and rejects Bory's specific name *pirenula*. He notes the prevalence of this organism in the tartar of patients on low diet for several months and states that it forms the greater part of the mucus coat on the tongue of persons with disturbed digestion. Neither Mandl (1838–1845), nor Moquin-Tandon (1860), nor any subsequent investigator of the flagellates of the human mouth, seems to have noted the resemblance of Müller's species to *Trichomonas;* and Davaine (1854, 1860), Leuckart (1863), and all subsequent investigators of the flagellates of the human digestive tract seem to have overlooked Müller's discovery and its possible relation to the organisms with which they were dealing.

Since only one flagellate from the human mouth has thus far been definitely identified in the course of modern investigation of the

microfauna of that habitat, it is obvious that Müller (1773), Mandl (1838–1845), and Moquin-Tandon (1860) may have been dealing with that flagellate.

However, Müller's figures are so imperfect and his description so meagre that there is no certainty whatever that he had a *Trichomonas* and there is still less a clue to the specific identity of the moving organism he discovered. It may well be that with additional investigation more than one kind of flagellate will be found in the mouth.

In view of the lack of critical evidence we reject Müller's *Cercaria tenax* as unidentifiable with *Tetratrichomonas buccalis* Goodey and Wellings and as an indeterminable species. The specific name *tenax* is therefore not available and *Trichomonas buccalis* (Goodey and Wellings) becomes the correct name for the trichomonad flagellate of the mouth.

It is possible that the trichomonad of the lung and that of the mouth belong to the same species, but this is not as yet proved and *Trichomonas pulmonalis* Schmidt (1895) is so inadequately characterized as to be unidentifiable. As described it cannot be distinguished from *T. intestinalis* and *T. vaginalis,* and the specific name *pulmonalis* should be allowed to lapse as indeterminable.

One factor complicating the status of these several species is the question as to the number of flagella which in the earlier accounts (Davaine, 1854, 1860, and others) was reported as one, and in others, such as Kunstler (1898), as varying (in *Trichomonas* of the rat) from 4–8–10 up to 15, while in recent years Wenyon and O'Connor (1917) admit that there are forms with three, four, and five flagella.

The uncertainty as to the number of flagella in both *T. vaginalis* and *T. hominis* is shown by Doflein's (1916) cautious treatment of this matter. He regards three flagella as typical and four as possibly resulting from the detachment of the marginal filament of the undulating membrane, and a resulting "*Trichomastix*" condition which has four free flagella, one of them trailing. This conclusion appears to me incorrect, after a review of the literature, and careful examination of my own preparations and cultures and of other preparations secured from Drs. Lynch, E. L. Walker, and F. G. Haughwout. In every instance where the number of flagella can be accurately determined we have unquestionably found four anterior flagella and an undulating membrane with marginal filament present in both *T. hominis* and *T. vaginalis.*

There is a tendency for the flagella of *Trichomonas* to adhere to one another in a single strand, parting only distally. Often only three

flagella adhere in this bundle and the fourth is thrown down at one side. This makes determination difficult and leads to the acceptance of three as the total number of anterior flagella present. Lynch (1915) found four flagella on *T. hominis* in culture. Wenyon and O'Connor (1917) state that four flagella were found by them in all cases of *T. intestinalis* except on two "occasions," when three and five respectively were found. Kunstler (1884) and Lynch (1915) found four flagella in *T. vaginalis.*

There is so much discrepancy in the results of different investigators as to the number of flagella that one can not be dogmatic and deny the existence of species in man with only three flagella. We affirm that the most critical evidence indicates that *T. hominis* and *T. vaginalis* have four flagella, and that no report of a *Trichomonas* with three flagella from man can be accepted as critical until verified on a number of living flagellates restrained for observation or fixed by fumes of osmic acid, 10 per cent formalin, or Gram's iodine, and after analysis of a number of carefully stained individuals.

Four anterior flagella have also been found on *Tetratrichomonas buccalis* by Goodey and Wellings (1917). The number of flagella on the *Trichomonas* of the respiratory tract has not been determined.

Ohira and Nogouchi (1917, February) in a paper published prior to that of Goodey and Wellings (1917, July) name the *Trichomonas* of the mouth *Tetratrichomonas hominis,* but the specific name *hominis* is preoccupied in *Trichomonas* by *T. hominis* Davaine. This necessitates the use of *buccalis* Goodey and Wellings for this flagellate.

Tetratrichomonas Parisi 1910

This was established by Parisi (1910) as a subgenus of *Trichomonas* for a parasitic species (*T. prowazeki*) from *Salamandra,* which had four instead of three free anterior flagella. He assigns to *Trichomonas sensu strictu* these species with only three anterior flagella. Goodey and Wellings (1917) raise *Tetratrichomonas* to generic status.

The genus *Trichomonas* was established by Donné (1837) with *vaginalis* as the only, and therefore the type, species. He did not determine the number of flagella in *T. vaginalis* as four, but later investigators have done this. It follows from this that the genus *Trichonomas* is founded on a species with four flagella, that *Tetratrichomonas* is a synonym of *Trichomonas,* and that its type species, *T. prowazeki,* belongs in *Trichomonas.*

In view of the fact that the number, disposition, and specialization of the flagella have been the basis of differentiation in the Polymastigina and that the genera of the order are distinguished by their flagellar complexes, it seems to be necessary to establish a new generic name for those species of *Trichomonas sensu latu* which have only three anterior free flagella. It is necessary to reserve *Trichomonas* for species with four flagella.

We therefore propose the new generic name *Tritrichomonas* for all trichomonad flagellates with the following diagnostic characters:

Tritrichomonas nom. gen. nov.

Polymastigote flagellates with three free anterior flagella arising from an anterior centroblepharoplast. This centroblepharoplast gives rise to a posteriorly directed, laterally attached flagellum forming the marginal filament in the sinistral slightly spiral undulating membrane terminating distally in a short free flagellum; it also gives rise to the parabasal body along the base of the undulating membrane, and to the axostyle; cytostome not heavily margined with a deeply staining fibril. We designate the well-known *Trichomonas augusta* Alexeieff as the type species of *Tritrichomonas*.

The Genus Giardia

Giardia Kunstler (1882), pp. 347–349.
Giardia Kunstler (1883), pp. 52–54, pl. 2, figs. 1–4.
Dimorphus Grassi (1879), pp. 445–448. Nec *Dimorphus* Haller, 1878 (Arachnida); nec *Dimorpha* Jurine 1807 (Hymenoptera); nec *Dimorphus* Gray 1844 (Mollusca): Hodgson 1841 (Aves).
Megastoma Grassi (1881), pp. 575–580. Nec Megerle von Mühlfeld (1818) (Mollusca); Blainville 1825? (Mollusca); Swainson 1837 (Aves); Costa 1850 (Pisces).
Lamblia Blanchard (1888), pp. 18–19.
Giardia Kunstler, Alexeieff (1914), pp. 210–212, figs. 4, 5.

The first description of a species now included in this genus was that of Lambl (1859 *nec* 1875) of *Cercomonas intestinalis* from mucus stools of a child. His species is plainly not to be included in the genus *Cercomonas*. Davaine (1875) described a species belonging to *Giardia* from the rabbit as *Hexamita duodenalis*, evidently in ignorance of Lambl's account, but his species can not be allocated in *Hexamitus*.

Grassi (1879) described as *Dimorphus muris* a species belonging to *Giardia* from *Mus* and *Arvicola*, and later (1881) from man and the cat. *Dimorphus* is, however, a homonym with *Dimorphus* Haller

(1878) arachnoid. In his later paper Grassi changes the name from *Dimorphus muris* to *Megastoma entericum,* since "la denominazione *Dimorphus* potrebbe dar luogo ad un equivoco e l'altra muris ora e diventata insufficiente." *Megastoma,* as Blanchard (1888) indicated when he proposed the new generic name *Lamblia,* had already been preoccupied in no less than four instances cited above.

Prior to Blanchard's (1888) erection of *Lamblia* the genus *Giardia* had been established by Kunstler (1882, 1883*a, b*) for a species from the intestine of tadpoles. The human species is a member of this genus *Giardia* as Alexeieff (1914) has shown. Doflein's (1916, p. 628) objection to *Giardia* is in reality an objection to the Code of Nomenclature and to the just ethical claims of priority on the part of Kunstler and Alexeieff, and to sound morphology as applied to classification.

The generic term *Lamblia* is, therefore, as Alexeieff (1914) has recently shown, not tenable, and *Giardia* Kunstler must be substituted for it on the grounds of priority if due regard is had to the rules of nomenclature. This was regrettable from the fact that the term *Lamblia* had become entrenched by the custom of many compilers in both biological and medical literature, especially in textbooks and general treatises.

Some of the grounds for the inclusion of *Lamblia* (*Megastoma*) in *Giardia* were noted by Grassi and Schewiakoff (1888) when, in their discussion of the evident close relationships which they clearly recognized of the two genera, *Megastoma* Grassi and *Giardia* Kunstler, they based their rejection of *Giardia* upon the apparent absence of the cytosome, which was not demonstrated in Kunstler's (1883) figures or mentioned in his earlier discussion (1882, 1883).

However, in Kunstler and Gineste's (1907) later paper this structure is definitely described and figured in *Giardia alata,* sp. nov., also from the tadpole and probably the same species as *G. agilis.* In any event *Giardia* is recognized by its author as possessing the very organ whose apparent absence led to the generic recognition of *Megastoma* and of its successor *Lamblia.*

In 1907 Kunstler and Gineste discuss the synonymy of the three genera, *Megastoma, Giardia,* and *Lamblia,* and although they recognize the equivalence of the organelles of *Giardia* and *Lamblia,* they conclude, erroneously we believe, that both genera should be maintained "à la fois" although the only differences they can find are in proportions and size, distinctions which in the general experience of protozoologists are valid only for the separation of species.

The recent investigation by Alexeieff (1914) of the finer structure of *Giardia* and our own more extended investigation (Kofoid and Christiansen, 1915 *a* and *b*) bring convincing proof of morphological similarity of the species referred to *Giardia* to those heretofore included in *Lamblia*. They have the same organelles in similar relationships. Their differences consist solely in contour, shape, proportion, size, hosts, and shape of the parabasal bodies, and are valid only to separate species. The species of *Giardia* and *Lamblia* should therefore all be placed in one genus, as in the case of the various species of *Trichomonas* and *Trypanosoma*. By the accepted laws of nomenclature *Giardia* has priority.

The several species which have been described, especially by Bensen (1908), are based on slight differences in form and proportions, and Noc (1908) raises the question as to the validity of these distinctions. After our experience with the material which we have examined, which includes five species, *Giardia alata, G. muris, G. microti, G. duodenalis*, and *G. enterica*, and a wide experience with the genus *Trichomonas*, we are inclined to regard Noc's skepticism as not well grounded. One learns to detect those differences in size and proportions, due to movement and growth, and to recognize others, usually a group of characters, which constitute the specific distinctions, such for example as those between *G. muris* and *G. microti*. We believe therefore that Bensen (1908) was correct in making distinctions though it may be that not all his species will stand the test of fuller examination, and that not all his distinctive characters will prove valid.

This matter is one of more than restricted interest for it impinges, on the one hand, on the general biological question as to how far specificity of parasites to particular hosts obtains, and on the other hand on the very practical question as to whether or not rodents, especially mice, rats, and Belgian hares, are sources of infection to man, for these flagellate parasites, in mice and men at least, are often associated with chronic intestinal disturbances (Noc, 1909; Rodenwaldt, 1912; Jollos, 1913; Kofoid and Christiansen, 1915). If the species are distinct, it is less probable that rodents serve as sources for human infection. If these so-called specific differences are illusory, on the other hand, the dangers from rodents as carriers and sources are greatly enhanced.

There remains the further problem which experiment only can solve as to whether or not the same species of flagellate may pass through a series of host species and assume in each, or in certain

groups of hosts, a given set of so-called species characters, and another set in another species or group. The fact that *G. muris* occurs in both *Mus* and *Peromyscus*, and possibly also in the rat, without noticeable modification is an indication of the fixity of at least this species under different conditions as to hosts. Noc (1908) states that he finds in man *Lamblia* which exhibit the whole range in modifications described by Bensen for his species. It is important to learn in how far Noc's conclusions are critical, in how far the resemblances are superficial, and in how far certain combinations of size, proportion, and structure distinguish the species thus far described, and in how far these are correlated with predominant relations to certain species as hosts.

The determination of the correct specific name for the *Giardia* from man is entangled not only with the problem of the specific distinctness of the species of *Giardia* in other hosts than man, especially in rodents, but also with matters of synonymy and priority under the Code of Nomenclature.

In our present opinion the morphological evidence indicates that the several species normal to rodent hosts differ from that in man, and that the *Giardia* of man should receive a separate specific designation. Fantham and Porter's (1916*b*) experimental inoculation of mice by the *Giardia* from man does not necessarily invalidate the specific distinctness of the species of *Giardia* normally present in mice from that found in man.

The first *Giardia* to be described was that from man by Lambl (1859) as *Cercomonas intestinalis*, but this specific name is preoccupied in *Cercomonas* by Diesing's (1850) transfer of *Bodo intestinalis* Ehrbg. to *Cercomonas*, which this reviser treated as a subgenus of *Bodo*.

Davaine (1875) described *Heramita duodenalis* from the rabbit. This also is a *Giardia* but the species in the rabbit is distinct from that in man and *duodenalis* is therefore not available for the species from man. Grassi (1881) proposed the name *Megastoma entericum* for the flagellates belonging to *Giardia* which he had found in *Mus*, *Arvicola*, the cat, and man. In 1879 he had given the name *Dimorphus muris* to the species from *Mus* and *Arvicola* and in 1881 proposed *M. entericum* as a substitute with wider application. Since the species in the mouse is distinct from that in man the name *Giardia muris* is applicable only to the former. No data are available as to the status of the form in *Arvicola* and infection in the cat is presumably derived from that in mice. By elimination the specific name *Giardia enterica*

(Grassi) becomes available for the *Giardia* from man in place of *intestinalis* (Lambl).

The name *Giardia lamblia* Stiles (in litt.) proposed in Kofoid and Christiansen (1915*b*, p. 28) as a substitute for the preoccupied *intestinalis* becomes therefore a synonym of *G. enterica.*

SUMMARY

1. *Cercomonas* is not as yet proved to be a human intestinal parasite, but coprophilic species of this genus occur in stale stools or in stool cultures. *Cercomonas* of clinical literature is probably *Chilomastix.* Flagellate stages of *Craigia* may have been reported as *Cercomonas.*

2. *Cercomonas hominis* Davaine (1860) was a complex of two species. His var. *A*, named *Cercomonas davainei* by Moquin-Tandon (1860), is *Chilomastix.* The correct name for *Chilomastix* is not *C. mesnili* (Wenyon) but *C. davainei* (Moquin-Tandon).

3. Davaine's *Cercomonas hominis* var. *B* is the human intestinal *Trichomonas,* the correct name of which is *T. hominis* (Davaine). *Cercomonas obliqua* Moquin-Tandon (1860), *Trichomonas intestinalis* Leuckart (1879), and *T. confusa* Stiles (1902) are synonyms of *T. hominis.*

4. The type species of *Trichomonas* Donné (1837) has four free anterior flagella, hence *Tetratrichomonas* Parisi 1910 is a synonym of *Trichomonas. Trichomonas hominis* from the intestine and *T. buccalis* from the mouth also have four flagella.

5. A flagellate of the mouth was first described by O. F. Müller (1773) as *Cercaria tenax,* but the description and figure are too meagre to permit re-identification. *Tetratrichomonas buccalis* Goodey and Wellings (1917) should be transferred to *Trichomonas.*

6. A new genus *Tritrichomonas* is established for trichomonad flagellates with three anterior free flagella with *T. augusta* Alexeieff as type species.

7. *Giardia enterica* (Grassi) 1881 has priority over *Lamblia intestinalis* (Lambl) Blanchard 1888, and over *Giardia lamblia* Stiles 1915.

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LITERATURE CITED

Alexeieff, A.

1914. Notes protistologiques. Zool. Anz., 44, 193–213, 4 figs. in text.

Barlow, N.

1915. Craigiasis. Am. Jour. Trop. Med., 2, 680–702, pl. 26.

Bensen, W.

1908. Bau und Arten der Gattung *Lamblia*. Zeitschr. f. Hyg. u. Infekt., 61, 109–114, 6 figs. in text.

Blanchard, R.

1888. Remarques sur le megastome intestinal. Bull. Soc. Zool. France, 13, 18–19.

Brumpt, E.

1912. Côlite à *Tetramitus Mesnili* (Wenyon 1910) et Côlite *Trichomonas intestinalis* Leuckart 1879. Bull. Soc. Path. Exot., 5, 725–730, 3 figs. in text.

1913. Précis de Parasitologie. Preface de M. le professeur R. Blanchard; ed. 2 (Paris, Masson), pp. xxiv + 1011, 4 pls., 698 figs. in text.

Bory de Saint Vincent, J. B. G. N.

1824. *Virgulina* in "Hist. nat. des vers, etc." *in* Encyle. Méth., 3, Zoophytes.

Castellani, A.

1918. Alcune osservazione sulla etiologia, diagnose e cura della dysenteria. Ann. Med. Nav. e Colon., 24, 52–72, 3 figs. in text.

Chalmers, A. J., and Pekkola, W.

1917. *Chilomastix mesnili* (Wenyon, 1910). Ann. Trop. Med. Paras., 11, 213–264, pls. 6, 7, 1 fig. in text.

Chace, A. F., and Tasker, A. N.

1917. The diagnosis and treatment of flagellate diarrheas. Jour. Am. Med. Assoc., 68, 1528–1531, 1 fig. in text.

Christie, W. L.

1915. Further investigations into latent dysentery and intestinal parasitism in Sarawak, Borneo. Brit. Med. Jour., 1915, ii, 89–90.

Craig, C. F.

1911. The parasitic amoebae of man. (Lippincott, Philadelphia), pp. x + 253, 30 figs. in text.

Cutler, D. W., and Williamson, R.

1919. A note on the Protozoa of the intestine. Jour. Roy. Army Med. Corps, 33, 262–266.

Davaine, C.

1854. Sur les animalcules infusoires trouvés dans les selles de malades atteints du cholera et d'autres affections. Compt. Rend. Soc. Biol., Paris, (2), 1, 129–130.

1860. Traitê des Entozoaires et des maladies vermineuses de l'homme et des animaux domestiques; ed. 1 (Paris, Baillière et Fils), pp. xix + xcii +838 pp., 57 + 31 figs. in text.

1875. Monadiens. Dict. encycl. d. sci. méd. sér. 2, 9, 115–130.

Diesing, K. M.,

1850–1851. Systema helminthum (Vindibonae, Braumüller), 1, pp. xiii + 679.

Doflein, F.
1916. Lehrbuch der Protozoenkunde; ed. 4 (Leipzig, Fischer), pp. xv + 1190, 1198 figs. in text.

Donné, A.
1837. Recherches microscopiques sur la nature des mucus et la matière des divers écoulemens des organes genito-urinaires chez l'homme et chez la femme (Paris, Chevalier), pp. iv + 71, pl. 1.

Dujardin, F.
1841. Histoire naturelle des Zoophytes. Infusoires, comprenant la physiologie et la classification de ces animaux, et la manière de les étudier à l'aide du microscope (Suites à Buffon, Roret, Paris), pp. xii + 684, 22 pls.

Fantham, H. B.
1916. Remarks on the nature and distribution of the parasites observed in the stools of 1305 dysenteric patients. Lancet, 1916, i, 1165–1166.

Fantham, H. B., and Porter, A.
1916*a*. The pathogenicity of *Giardia* (*Lamblia*) *intestinalis* to men and to experimental animals. Brit. Med. Jour., 1920, ii, 139–141.
1916*b*. Notes on certain Protozoa which may be found in cases of dysentery from the Mediterranean war zone. Proc. Camb. Phil. Soc., 18, 184–188.

Goodey, T., and Wellings, A. W.
1917. Observations on *Entamoeba gingivalis* from the human mouth with a note on the trichomonad flagellate *Tetratrichomonas buccalis*, n. sp. Parasitology, 9, 537–559, pls. 20–22.

Grassi, B.
1879. Die protozoi parassiti e specialmente di quelli che sono nell'uomo. Gazz. Med. Ital. Lomb., 39, 445–448.
1881. Di un nuovo parasite dell'uomo *Megastoma entericum* (mihi). Gazz. degli Ospitali, Milano, 2, 575–580.

Grassi, B., and Schewiakoff, W.
1888. Beitrag zur Kenntnis des *Megastama entericum*. Zeitschr. f. wiss. Zool., 46, 143–154, pl. 15.

Guastalla, N.
1919. Flagellaten im menschlichen Darm. Beitrag zur Kenntniss der Infusorienenteritis. Wien. klin. Woch., 22, 1560–1563.

Hartmann, M., and Chagas, C.
1910. Flagellaten-Studien. Mem. Inst. Oswaldo Cruz., 2, 64–126, pls. 4–9, 3 figs. in text.

Janowski, W.
1897. Ueber Flagellaten in den menschlichen Fäces und über ihre Bedeutung für die Pathologie des Darmkanals. Zeitschr. f. klin. Med., 31, 442–492, pls. 4–5.

Kunstler, G.
1882. Sur cinque protozoaires nouveaux. Compt. Rend. Acad. Sci. Paris, 95, 347–349.
1883*a*. Recherchés seu les infusoires parasites. Sur quinze Protozoaires nonveaux. *Ibid.*, 97, 755–757.
1883*b*. Histoire naturelle des infusoires parasites. Ann. Sci. Nat. Bordeaux et du Snd-Onest, 3, 45–54, pls. 1, 2.
1884. *Trichomonas vaginalis* Donné. Jour. de micrographie, 1, 317–331, pls. 5, 6.

Kunstler, J., and Gineste, C.
1907*a*. *Giardia alata,* n. sp. Compt. Rend. Acad. Sci. Paris, **144**, 441–443.
1907*b*. *Megastoma, Lamblia* ou *Giardia?* Bull. Soc. Zool. France, **32**, 28–32.
Kofoid, C. A.
1920. Intestinal protozoan infections in United States army troops. Trans. Amer. Gastroenterol. Assoc. *In press.*
Kofoid, C. A., and Christiansen, E. B.
1915*a*. On *Giardia microti,* sp. nov., from the meadow mouse. Univ. Calif. Publ. Zool., **16**, 23–29, 1 fig. in text.
1915*b*. On binary and multiple fission in *Giardia muris* Grassi. *Ibid.*, 30–54, pls. 5–8, 1 fig. in text.
Lambl, W.
1859. Mikroskopische Untersuchungen der Darmexcrete. Beitrag zur Pathologie des Darms und zur Diagnostik am Krankenbette. Vierteljahrschr. prakt. Heilk., **61**, 1–58, pls. 1–4, figs. 1–13 in text.
1875. *Cercomonas* et *Echinococcus* in hepate hominis. Med. Vestnk., St. Petersburg.
Ledingham, J. C. G., Penfold, W. J., and Woodcock, H. M.
1915. Recent bacteriological experiences with typhoidal diseases and dysentery. With notes on the protozoan parasites by Woodcock, H. M. Brit. Med. Jour., **1916**, ii, 704–711, 4 figs. in text.
Leuckart, R.
1863. Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten. Ein Hand- und Lehrbuch für Naturforscher und Aertze (Leipzig, Winter), pp. viii + 766, 268 figs. in text.
1879. *Idem,* ed. 2, 1, Lief. 1, pp. viii + 336, 130 figs. in text.
Lynch, K. M.
1915*a*. Trichomoniasis of the vagina and the mouth. Cultivation of the causative organism and experimental infection. Am. Jour. Trop. Dis. and Preventive Med., **3**, 627–634.
1915*b*. Clinical and experimental trichomoniasis of the intestine with cultivation of the causative organism. New York Med. Jour., **101**, 886–889.
Mandl, L.
1838–1845. Anatomie microscopique (Paris, Baillière).
Matthews, A. J., and Smith, A. M.
1919. The intestinal protozoal infections among convalescent dysenterics examined at the Liverpool School of Tropical Medicine (Third report). Ann. Trop. Med. Parasit., **13**, 83–90.
Mello, Leitao.
1913. Flagellate dysentery in children. Brit. Jour. Children's Dis., **10**, 60–66.
Mouquin-Tandon, A.
1860. Éléments de zoologie médicale (Paris, Baillière et Fils), pp. xvi + 428, 122 figs. in text.
1861. Elements of medical zoology. Translated and edited by R. T. Hulme (London, Baillière), pp. xvi + 423, 124 figs. in text.
Müller, O. F.
1773. Vermium terrestrium et fluviatilium, seu animalium infusorium, helminthocorum et testaceorum, non marinorum, succincta historia (Havniae et Lipsiae, Heineck et Taber), v. 1 (1), pp. 30 + 135; v. 1 (2), pp. 72 + 8; v. 2, pp. xxxvi + 214 + 10.

1776. Zoologiae Danicae prodromus, seu animalium Daniae et Norvegiae indigenarum characteres, nomina et synonyma imprimis popularium (Havniae, Hallageriis), pp. xxii + 274.

1786. Animalcula infusoria fluviatila et marina, quae detexit, systematica descripsit et ad vivum delineari curavit. Cura Othonis Fabricii (Havniae, Mölleri), pp. lvi + 362, 50 pls.

Neveu-Lemaire, M.

1908. Précis de parasitologie humain. Maladies parasitaires dues à des végetaux et des animaux (Paris, Rudeval), ed. 4, pp. v + 712, 391 figs. in text.

Noc, F.

1908. Observations sur le cycle évolutif de *Lamblia intestinalis*. Bull. Soc. Path Exot., 2, 93–97.

Parisi, B.

1910. Su alcuni flagellati endoparassiti. Arch. f. Prot., 19, 232–238, pl. 14.

Porter, A.

1918. A survey of the intestinal Entozoa, both protozoal and helminthic observed among natives in Johannesburg from June to November, 1917. Publ. So. African Inst. Med. Research, no. 11, 58 pp., 2 pls.

Prentiss, C. W.

1914. Infection with the *Cercomona hominis*. New Mexico Med. Jour., December, 1914. Reprint, 5 pp.

Railliet, A.

1886. Éléments de zoologie medical et agricole (Paris, Asselin et Houzeau), pp. xv + 1053, 705 figs. in text.

1895. Traité de zoologie médicale et agricole (Paris, Asselin et Houzeau), ed. 2, pp. xv + 1803, 892 figs. in text.

Sangiorgi, G.

1919. Dissenterie in Albania. Pathologica, 21, 141–144.

Simon, S. K.

1918. *Cercomonas* diarrhea and its management. Southern Med. Jour., 11, 414–417.

Smithies, F.

1918. The frequency of protozoic enterocolitis in the Middle West, clinical manifestations, diagnosis and treatment. Am. Jour. Med. Sci., 146, 173–184.

Stiles, C. W.

1902. The type species of certain genera of parasitic flagellates, particularly Grassi's genera of 1879 and 1881. Zool. Anz., 25, 689–695.

Verdun, P.

1907. Précis de parasitologie humaine. Parasites animaux et végetaux (les bactériees exceptées) (Paris, Doin), pp. iii + 727, 4 pls., 310 figs. in text.

Wenyon, C. M.

1910a. A new flagellate (*Macrostoma mesnili*, n. sp.) from the human intestine, with some remarks on the supposed cysts of *Trichomonas*. Parasitology, 3, 210–216, pl. 16.

1910*b*. Some observations on a flagellate of the genus *Cercomonas*. Quart. Jour. Micr. Sci., n.s., 55, 241–260, 19 figs. in text.

Wenyon, C. M., and O'Connor, F. W.

1917. Human intestinal Protozoa in the Near East (London, Bale), pp. 218, 4 pls.

11. A Study of the Races of the White-Fronted Goose (*Anser albifrons*) occurring in California, by H. S. Swarth and Harold C. Bryant. Pp. 209-222, 2 figures in text, plate 13. October, 1917 ..... .15

12. A Synopsis of the Bats of California, by Hilda Wood Grinnell. Pp. 223-404, plates 14-24, 24 text figures. January 31, 1918 ..... 2.00

13. The Pacific Coast Jays of the Genus *Aphelocoma*, by H. S. Swarth. Pp. 405-422, 1 figure in text. February 23, 1918 ..... .20

14. Six New Mammals from the Mohave Desert and Inyo Regions of California, by Joseph Grinnell. Pp. 423-430.

15. Notes on Some Bats from Alaska and British Columbia, by Hilda Wood Grinnell. Pp. 431-433.
Nos. 14 and 15 in one cover. April, 1918 ..... .15

16. Revision of the Rodent Genus *Aplodontia*, by Walter P. Taylor. Pp. 435-504, plates 25-29, 16 text figures. May, 1918 ..... .75

17. The Subspecies of the Mountain Chickadee, by Joseph Grinnell. Pp. 505-515, 3 text figures. May, 1918 ..... .15

18. Excavations of Burrows of the Rodent *Aplodontia*, with Observations on the Habits of the Animal, by Charles Lewis Camp. Pp. 517-536, 6 figures in text. June, 1918 ..... .20
Index, pp. 537-545.

Vol. 18. 1. Mitosis in *Giardia microti*, by William C. Boeck. Pp. 1-26, plate 1. October, 1917 ..... .35

2. An Unusual Extension of the Distribution of the Shipworm in San Francisco Bay, California, by Albert L. Barrows. Pp. 27-43. December, 1917. .20

3. Description of Some New Species of *Polynoidae* from the Coast of California, by Christine Essenberg. Pp. 45-60, plates 2-3. October, 1917 ..... .20

4. New Species of *Amphinomidae* from the Pacific Coast, by Christine Essenberg. Pp. 61-74, plates 4-5. October, 1917 ..... .15

5. *Crithidia euryophthalmi*, sp. nov., from the Hemipteran Bug, *Euryophthalmus convivus* Stål, by Irene McCulloch. Pp. 75-88, 35 text figures. December, 1917 ..... .15

6. On the Orientation of *Erythropsis*, by Charles Atwood Kofoid and Olive Swezy. Pp. 89-102, 12 figures in text. December, 1917 ..... .15

7. The Transmission of Nervous Impulses in Relation to Locomotion in the Earthworm, by John F. Bovard. Pp. 103-134, 14 figures in text. January, 1918 ..... .35

8. The Function of the Giant Fibers in Earthworms, by John F. Bovard. Pp. 135-144, 1 figure in text. January, 1918 ..... .10

9. A Rapid Method for the Detection of Protozoan Cysts in Mammalian Faeces, by William C. Boeck. Pp. 145-149. December, 1917 ..... .05

10. The Musculature of *Heptanchus maculatus*, by Pirie Davidson. Pp. 151-170, 12 figures in text. March, 1918 ..... .25

11. The Factors controlling the Distribution of the Polynoidae of the Pacific Coast of North America, by Christine Essenberg. Pp. 171-238, plates 6-8, 2 figures in text. March, 1918 ..... .75

12. Differentials in Behavior of the Two Generations of *Salpa democratica* Relative to the Temperature of the Sea, by Ellis L. Michael. Pp. 239-298, plates 9-11, 1 figure in text. March, 1918 ..... .65

13. A Quantitative Analysis of the Molluscan Fauna of San Francisco Bay, by E. L. Packard. Pp. 299-336, plates 12-13, 6 figs. in text. April, 1918 ..... .40

14. The Neuromotor Apparatus of *Euplotes patella*, by Harry B. Yocom. Pp. 337-396, plates 14-16. September, 1918 ..... .70

15. The Significance of Skeletal Variations in the Genus *Peridinium*, by A. L. Barrows. Pp. 397-478, plates 17-20, 19 figures in text. June, 1918 ..... .90

16. The Subclavian Vein and its Relations in Elasmobranch Fishes, by J. Frank Daniel. Pp. 479-484, 2 figures in text. August, 1918 ..... .10

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Nos. 17 and 18 in one cover. January, 1919 ..... .35
Index in preparation.

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2. The Pteropod *Desmopterus pacificus* (sp. nov.), by Christine Essenberg. Pp. 85–88, 2 figures in text. May, 1919 ..... .05

3. Studies on *Giardia microti*, by William C. Boeck. Pp. 85-136, plate 1, 19 figures in text ..... .60

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10. A New Morphological Interpretation of the Structure of *Noctiluca*, and its Bearing on the Status of the Cystoflagellata (Haeckel), by Charles A. Kofoid. Pp. 317–334, plate 18, 2 figures in text. February, 1920 ..... .25

11. The Life Cycle of *Echinostoma revolutum* (Froelich), by John C. Johnson. Pp. 338–388, plates 19–25, 1 text figure. May, 1920 ..... .60

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2. Studies on the Parasites of the Termites. II. On *Trichomitus termitidis*, a Polymastigote Flagellate with a Highly Developed Neuromotor System, by Charles Atwood Kofoid and Olive Swezy. Pp. 21–40, plates 3–4, 2 figures in text. July, 1919 ..... .25

3. Studies on the Parasites of the Termites. III. On *Trichonympha campanula* Sp. Nov., by Charles Atwood Kofoid and Olive Swezy. Pp. 41–98, plates 5–12, 4 figures in text. July, 1919 ..... .75

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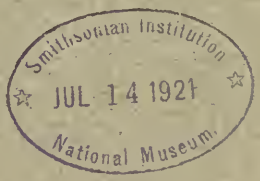
ON THE FREE, ENCYSTED, AND BUDDING STAGES OF COUNCILMANIA LAFLEURI, A PARASITIC AMOEBA OF THE HUMAN INTESTINE

BY
CHARLES ATWOOD KOFOID AND OLIVE SWEZY



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ON THE FREE, ENCYSTED, AND BUDDING STAGES OF COUNCILMANIA LAFLEURI, A PARASITIC AMOEBA OF THE HUMAN INTESTINE*

Smithsonian Institution
JUL 14 1921
National Museum

BY
CHARLES ATWOOD KOFOID AND OLIVE SWEZY

CONTENTS

	PAGE
Introduction	169
Material and methods	171
Activities	171
The free amoeba	173
The encysted phases	175
Dimensions	180
Budding and escape of amoebulae	181
Occurrence	183
Culture and inoculation	185
Viability of cysts	185
Distinctions between *Councilmania lafleuri* and *Endamoeba coli*	186
Summary	187
Literature cited	188

INTRODUCTION

The perplexing confusion which has long existed regarding the amoebae of the human digestive tract owes its origin and growth to a complex of causes, including the difficulties of securing and controlling fresh normal material, the existence of pathological, moribund, and abnormal stages in the parasitic organisms themselves, and the presence in man of multiple infections of molds, yeasts, flagellates, coccidia, and amoebae, with diverse races and phases in their life histories. The clinician and protozoologist must learn to distinguish these several organisms in their various aspects and avoid confusing them if therapy is to be critically applied, and morphology and life history are to be correctly interpreted.

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The intensive study of the protozoan infections of the human digestive tract incident to the prevalence of dysentery during the late war among troops on the Western, and especially the Near Eastern fronts, has clearly established the unexpectedly wide distribution of amoebic infections in man.

In the case of these amoebae the confusion is increased by the existence of no less than six different valid species of amoebae in human stools and of size races within some, if not all, of these. These are the three common species: *Endamoeba dysenteriae* (Councilman and Lafleur), *Endamoeba coli* (Lösch), *Endolimax nana* (Wenyon and O'Connor), and the rare *Dientamoeba fragilis* (Dobell and Jepps). We have recently found a clearly established case of human infection with a fifth amoeba called *Entamoeba muris* (Grassi) by Brug (1919), which Brug and ourselves find to be a normal parasite of wild and culture rats. Under *Endolimax nana* we include tentatively the form known as the "iodine body" which we regarded (see Kofoid, Kornhauser, and Swezy, 1919) as the large glycogen-bearing race of *Endolimax nana.*

In connection with *Endamoeba coli* some differences of opinion and of observation still persist with regard to its activities, structure, and life history. Specifically these concern the appearance of the pseudopodia, the rapidity of formation of pseudopodia and of movement, the ingestion of red blood corpuscles, pathogenicity, the structure of the karyosome, and the formation of amoebulae by a process resembling budding from cysts in the stools. These debated points we hope will be cleared up by the evidence here presented that there is in man another amoeba hitherto undescribed and heretofore confused with *Endamoeba coli,* primarily because of its eight-nucleated cyst.

It is the purpose of this paper to describe this sixth species of amoeba from the human intestine, which may be present in enormous numbers, appears to have pathogenic capacities, and has apparently been passed over as *E. coli* because of its large size, its eight-nucleated cyst, and its high resistance to stains, all of which tend to obscure its distinctive characteristics.

We take pleasure in naming this amoeba *Councilmania lafleuri,* in recognition of the critical discovery in 1891 by these two investigators of the relation of the amoeba called by them *Amoeba dysentcriae* to dysentery and hepatic abscess, a landmark in the medical conquest of this widespread infection of man.

MATERIAL AND METHODS

The material upon which this study is based is derived from ten cases under observation for varying periods from July, 1920, to June, 1921. One case especially has been examined almost daily for over 120 days, the infection appearing, prior to treatment, in nearly every stool examined from this and some of the other cases under observation. Hundreds of fresh smears and over nine hundred prepared slides of both stained smears and sections have been available for study, as have also several warm stools with an abundance of free amoebae.

Our attention was first drawn to the species by its remarkable resistance to stain in the usual Schaudinn-iron haematoxylin treatment, the cysts remaining quite colorless while those of other intestinal amoebae were stained as usual. It was only after fixing in Schaudinn's fluid at 60° C. for several minutes that we were able to stain the cysts in iron haematoxylin.

We have been able to observe the living organism in the electric incubator and to secure preparations of all stages from the free amoeba to the eight-nucleated cyst and the escaping amoebulae, and are thus able to determine its distinctive characteristics.

We are deeply indebted to physicians and patients who have made this investigation possible, especially to Dr. C. L. McVey, of the staff of the University Infirmary, and to Dr. R. T. Legge, University Physician, and to students in their charge who have generously co-operated to make our observations possible. Acknowledgments are gratefully made for grants from Mrs. Margaret B. Fowler and an alumnus of the class of 1900 in support of this work.

ACTIVITIES

In warm liquid stools following a purge of magnesium sulphate, examined on the warm stage or electric incubator, the free amoebae predominate and are many of them very active. Some are rounded up and inactive and among them are probably precystic stages discharged prior to the early phases of encystment.

Amoebae in the active stages creep about with great rapidity. One elongated amoeba traversed five times its length in one minute and

its whole length in five seconds. There are frequent changes in direction of locomotion, due either to the swerving of the pseudopodium as a whole or to the origin of pseudopodia at new areas on the periphery. The nucleus generally lags in the posterior part of the body when the same general direction is maintained continuously for a time. The pseudopodia lie at or near the level of the substrate.

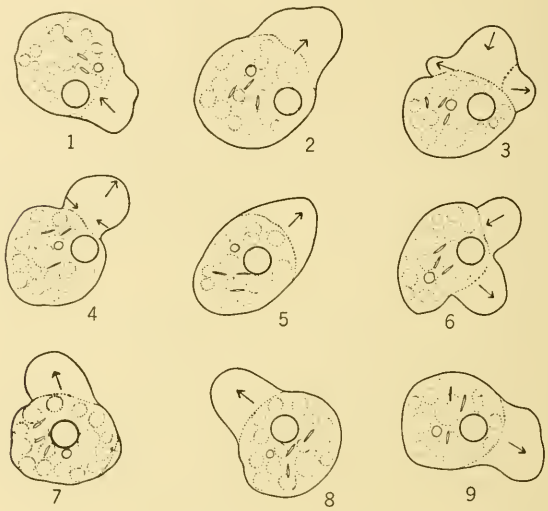

Fig. A. Free-hand sketches of nine successive phases of formation of pseudopodia in an active amoeboid stage of *Councilmania lafleuri* during one minute. × about 600.

There is generally a single pseudopodium in formation at one time. It is *perfectly clear* and appears to be composed wholly of ectoplasm. It is usually broad, from one-fourth to one-half the diameter of the body in width, though in elongated, traveling individuals it may rarely equal the width. In rounded-up individuals a slender pseudopodium one-fifth the diameter in width and as long as the radius may be thrown out. The tip of the pseudopodium is broadly rounded, rarely crenated or lobed, never pointed or bifureated. In mobile individuals the pseudopodium is quickly invaded by the granular, vacuolated endoplasm. Pseudopodia may be found at two, rarely three, areas on the

periphery at one time, but usually one of these is advancing and the others withdrawing.

One of the most noticeable features in connection with the pseudopodia of this amoeba is the expulsive suddenness of their formation. They are shot out almost instantaneously for the greater part of their length while the remaining extension and peripheral modifications proceed more leisurely. Invasion by the endoplasm is less rapid than pseudopodial formation. In the event of change of direction of locomotion the withdrawal of the pseudopodium is relatively very slow. The suddenness and rapidity of formation of the pseudopodia is the most striking feature of the free stage of this very active amoeba.

THE FREE AMOEBA

Plates 18–20, figures 1–17

The normal *free stage* of *Councilmania lafleuri* occurs in diarrheic and dysenteric stools and in liquid stools after a saline purge and sometimes in strands of bloody mucus on formed stools. It is rounded up in cold stools, and when extended becomes sluggish with the drop in temperature. In rare instances formation of pseudopodia may continue for as long as thirty minutes in ordinary smears without the warm stage.

The *ectoplasm* of *Councilmania* is most evident in the clear hyaline pseudopodia. Over the remainder of the body it forms a scarcely visible peripheral film. In life the ectoplasm of the pseudopodia appears wholly structureless. In stained preparations it is often somewhat chromophile in the pseudopodia in iron haematoxylin and destains slowly, exhibiting a dense homogeneous substance of fine texture quite distinct from the granular and vacuolated endoplasm (pl. 18, fig. 4). In some amoebae the ectoplasm destains wholly in the pseudopodia or in their peripheral parts. In rounded-up individuals the ectoplasm forms only a thin peripheral film, although in certain individuals which seem to belong to *Councilmania*, from their nuclear structure, there is a wide, vacuolated, clear zone in the periphery.

The pseudopodia of *Councilmania* differ from those of *Endamoeba coli* in being free from coarsely granular, vacuolated endoplasm, and from those of *E. dysenteriae* in being perhaps rather smaller, restricted as a rule, in the active state, to a smaller part of the periphery, and in the expulsive suddenness of their formation. They are also sometimes more chromophile.

The *endoplasm,* on the other hand, is coarsely vacuolated in the free stage and may be loaded with food vacuoles containing bacteria, red blood corpuscles, and occasionally a cyst of *Chilomastix* (pl. 19, fig. 6), or other particles from the faeces. This marked vacuolation and the great abundance of food contents of all sorts distinguish it at once from *Endamoeba dysenteriae,* but lead to confusion with *Endamoeba coli* in the absence of pseudopodia.

The food vacuoles and the fluid-filled ones which are scattered among them may crowd to the periphery of the body locally, or they may leave a narrow peripheral zone relatively free from such inclusions. They are moved about freely in the mobile endoplasm, as is the nucleus. The latter often lies on the very edge of the endoplasm.

The *nucleus* in the free stage presents different aspects, according to the degree of destaining and to phases of the mitotic cycle. The chromatin is distributed in the peripheral zone on the nuclear membrane and in a karyosome separated from the former by an intermediate clear zone. The peripheral chromatin is broken up into a relatively thin sheet (as compared with that of *Endamoeba coli*) of somewhat uniform particles flattened against the membrane and giving to it in some instances a crenate inner margin in optical section. Occasionally a larger particle is interpolated. This layer is not so thick as in *Endamoeba coli,* but is much thicker in the free amoebae than in the nuclei of the older cysts in *Councilmania,* and is also thinner in free amoebae when the karyosome is enlarged and mitosis is approaching.

The *karyosome* in the resting nucleus is generally somewhat excentric, as it is in *E. coli.* Not rarely it is quite excentric. It may be spheroidal or angular and has a very narrow, not always concentric, clear halo about it. This halo can not be found in many nuclei, especially those in which mitosis is approaching. In such nuclei the karyosome takes on the form seen in the cysts and has a much larger volume of chromatin, which breaks up into particles forming a sphere, ring, or reniform mass as the spireme stage approaches (pl. 19, figs. 7, 8).

The *intermediate* zone is free from distinct structural modifications. There may be a faint reticulum and occasionally a few minute chromatin granules in it in heavily destained preparations. We are unable, with our method of fixation and available eosins, to demonstrate in it any eosinophile granules such as Dobell (1920, pl. 2, figs. 32–34) finds in the amoeba which he regards as the free stage of his *Iodamoeba buetschlii.* Otherwise there is enough in common between free *Coun-*

cilmania and these figures of Dobell's to suggest the desirability of verification of his interpretation that the free amoeba assigned by him (his pl. 2, figs. 32–34) to his *Iodamoeba buetschlii* really belongs to that species rather than to *Councilmania*. Stools in which *Councilmania* is not found but which carry the encysted stage of *Iodamoeba* should be searched for free amoebae. No "*Iodamoeba*" has been found in either free or in encysted stages in the cases from which our free *Councilmania* have been derived.

There is usually only a single nucleus in the free amoeba. We have, however, found a number of amoebae with two nuclei each (pl. 19, fig. 8) with the karyosomes in the skein stage. They do not appear to be abnormal. In several instances in stools after prolonged treatment with antimony trioxide amoebae have been found in which no nucleus whatever can be detected. Many dead and abnormal amoebae were present in these stools.

THE ENCYSTED PHASES

Plates 20–22, figures 11–28

The precystic and encysted stages of this amoeba occur in non-diarrheic stools predominantly, free stages being rare and usually rounded up, moribund, and not reviving into activity on the warm stage when taken from stools recently cooled. These unencysted individuals represent either precystic individuals, those from the lower parts of the bowel which have had insufficient time to pass into the encysted phase, or more often moribund individuals, perhaps from higher levels in the bowel which, by reason of senescence or other physiological states, are not proceeding with encystment. The only activity aroused in a few instances in these individuals on warming is the slow protrusion or the withdrawal of broad shallow pseudopodia one-third to one-half of the circumference in width.

The *precystic phases* (pl. 19, figs. 6, 7) are uninucleate, rounded up, with cytoplasm relatively free from food inclusions. The nucleus does not as a rule differ from that in the free stage, has an enlarged, often excentric karyosome with less evidence of a clear zone about it, and considerable peripheral chromatin, and shows no indications of approaching mitosis.

The *encysted stage,* on the other hand, has a definite cyst wall, generally exhibits eight nuclei, each with large, often asymmetrical karyosome often centrally located, little peripheral chromatin, no

glycogen, and massed or scattered, somewhat pointed chromatin bodies. The latter may not be present.

The advanced cysts in the fresh stool examined by light from a Mazda lamp with blue glass screen have a grayish tone corresponding to the pale olive, light mineral, or pallid neutral gray of Ridgway's "Color Standards and Color Nomenclature." The thick hyaline wall of the cyst stands off the bacteria and in optical section its innermost

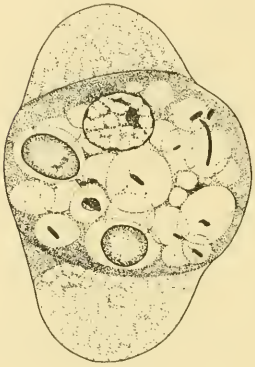

Fig. B

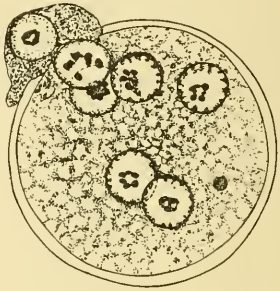

Fig. C

Fig. B. Free amoeba of *Councilmania lafleuri*, with food vacuoles containing bacteria and red blood corpuscles. Ectoplasm sharply separated from the endoplasm. Nucleus with excentric karyosomes and thin zone of peripheral chromatin. × 1600.

Fig. C. Budding cyst of *Councilmania lafleuri* with six nuclei remaining in the cyst and a seventh in a budding amoebula with chromophile cytoplasm. Nuclei with somewhat excentric karyosomes of the dispersed type and some granular peripheral chromatin forming on the nuclear membrane. × 1600.

zone has a dark brownish tinge. The nuclei are indistinct or faintly outlined. The chromatoidal bodies, when present, are somewhat more highly refractive than the cytoplasm.

The *cytoplasm* in the iodine-eosin stain shows more clearly a coarsely irregular alveolar structure, the chromatoidal bodies are more distinct, the nuclei and karyosomes become more evident, and the whole cyst assumes in time a modified chrome yellow tone. Occasionally remnants of food vacuoles and their contents are found in precystic stages, and rarely in later encysted phases.

The eight-nucleate encysted stage is preceded by the uninucleate, binucleate, and quadrinucleate stages, which are less abundant than those with eight nuclei in most stools, but may be found in larger numbers in liquid stools or in mucus strands on formed stools. Three successive divisions occur, resulting in two, four, and eight nuclei. These divisions are approximately synchronous in the one, two, and four nuclei at the successive mitoses, though slight irregularities in division rate or even an abnormal or moribund nucleus may occasionally be found. Occasionally a fourth mitosis in the cyst which would result in sixteen nuclei is indicated by the prophase conditon of the eight nuclei. In one instance a cyst with eleven nuclei was found. This last mitosis proceeds sometimes during budding.

In the uninucleate stage (pl. 20, fig. 11) the nucleus is pushed to the wall by the central vacuole, as are also the nuclei of the binucleate (pl. 20, fig. 12), and sometimes in the quadrinucleate (pl. 20, fig. 14) stages, while in the cyst with eight nuclei, and sometimes in the quadrinucleate ones, the vacuole has vanished and the nuclei are distributed through the cytoplasm (pl. 20, fig. 15). The nuclei in these early stages are much larger than in the later ones. The prevalence of mitotic phases and the nuclear conditions generally in the stages prior to the cyst with eight nuclei indicate that the three mitoses follow rapidly one after the other, though the predominance of the binucleate phase suggests that this is more prolonged than the quadrinucleate. Not all stools, even liquid ones, contain these stages, as a consequence, probably, of recurrent periods of multiplication in the bowel in the intervals between which such stages are rare or wanting.

There are eight *chromosomes* in *Councilmania lafleuri* clearly demonstrable at the metaphase, while *Endamoeba coli* has but six. An intradesmose connecting distinct chromatic polar masses is prominent in the prophase-metaphase period of mitosis, in the first and second mitoses especially, in *Councilmania*. It lies in contact with the nuclear membrane.

The term *intradesmose* is proposed for the chromophile strand between two polar centrosomes of the spindle, which lies on the *inner* face of the nuclear membrane, in contradistinction to the *paradesmose* of flagellates on the *outer* side of the nuclear membrane.

In the precystic stage neither glycogen vacuole nor chromatoidal bodies are present, but both develop shortly after encystment. The glycogen vacuole develops first. Many uninucleate cysts have this vacuole but no chromatoidal bodies, which appear later and usually

continue from the binucleate stage until even after eight nuclei are formed and budding begins.

The *"glycogen" vacuole* (pl. 20, figs. 11–13) is centrally located, and is relatively very large, in extreme instances filling seven-eighths of the diameter of the cyst (pl. 20, fig. 11). At first it is spheroidal, but is indented on one side by the enlarging nucleus. In the binucleate stage the nuclei at first lie close together, but later are found on opposite sides of the vacuole, which they deeply indent. In later stages the cytoplasm comes more and more to encroach upon the contents of the vacuole, appearing in optical section as ridges or tapering processes (pl. 20, fig. 14).

The structure of the contents of this vacuole in the fresh cysts and in those in the iodine-eosin stain is that of a homogeneous, undifferentiated substance, though in a few instances a faint reticular mesh could be detected within it. In preparations passed through aqueous solutions in staining the contents of the vacuole are entirely removed. Its substance does not stain brown readily, if at all in some stools, in iodine-eosin. It remains in such stools wholly unchanged for hours in this stain. In smears in Best's carmine and in celloidin sections in the same stain it remains unstained or at the most slightly tinged with pink. In a few stools only were we able to find cysts giving anything resembling a characteristic glycogen reaction in iodine. In one instance they were in a stool passed after three weeks of treatment with antimony trioxide and in another in stools after phenolax had been given. The stool contained a number of moribund and pathological amoebae, and we found a few advanced cysts in which the central vacuole stained a deep reddish brown and was somewhat broken up. This failure to react normally and readily to iodine leaves the nature of the substance in this vacuole somewhat problematical. It may be that the heavy cyst wall interferes with the typical glycogen reaction of the contents as it does with fixation and staining. The occurrence, period of existence in the life cycle, morphological relations, solubility in water, and stainability (in a few instances only), all indicate that it is glycogen, or some substance very similar to it.

The *chromatoidal bodies* (pl. 20, figs. 11–16; pl. 21, figs. 17, 19) are quite similar to those of *Endamoeba coli,* but somewhat stouter and less acicular. They first appear in the uninucleate, and especially in the binucleate cysts in the thin film of protoplasm surrounding the glycogen vacuole as numerous small flecks, blades, granules, or pointed fusiform bodies staining deeply in iron haematoxylin in the typical

fashion. As the glycogen mass decreases in size and finally vanishes they come to lie nearer the center of the cyst and may appear as a central bundle visible in fresh smears as a more highly refractive area, or in stained cysts as a fascicle of pointed splinters, or as scattered threads, or even in the last stages as small spheroidal masses (pl. 21, figs. 20, 22).

The chromatoidal substance appears to be involved in the structural and metabolic processes which result in the formation of the ridges, the opening of the pore, and the formation of the bud. This is shown in some instances by the radiation of a tripartite ridge from the end of the chromatoidal mass, or fascicle, nearest the cyst wall (pl. 21, fig. 19), or by the formation of the pore and bud at the end of the fascicle (pl. 22, fig. 23), and by the chromophile nature of the ridge (pl. 21, figs. 17–21), of the material in the pore (pl. 22, fig. 27), and of the young bud (pl. 22, figs. 23, 25, 27).

The *cysts* of *Councilmania* are quite variable in contour and are not predominantly spherical as in *Endamoeba coli*. In one hundred cysts in a fresh stool examined in iodine-eosin stain, twenty-four were spherical, forty spheroidal, twenty-two elongated or ellipsoidal, and twelve asymmetrical.

The *cyst wall* (pl. 20, figs. 12–16) is 0.8 to 1.5μ in thickness and when fully formed is laminated and triple contoured. It consists in optical section of a thin, outermost, sharp line, a middle, wide, clear zone, and an innermost, dark brownish, granular zone nearest to the cytoplasm. The clear zone is not always evident in the presumably younger cysts. The cyst wall is generally thicker than in *Endamoeba coli*.

The *structure of the nuclei* in the cysts is extraordinarily variable as a result of the prevalence of premitotic and postmitotic phases and of the fact that the karyosome is relatively large, sometimes asymmetrical and centrally located or slightly or even decidedly excentric, and somewhat subdivided. It often takes on a crescentic or reniform contour and is rarely seen in the cysts in the form of a single central, or excentric, spheroidal granule. In fresh stools, especially in those some of whose cysts are budding, the karyosome may have the form of a stout, reniform, or semicircular spireme with large chromomeres (pl. 21, figs. 17–22), or the chromomeres may be scattered throughout the nucleus (pl. 21, fig. 18).

The *nuclear membrane* is very thin but distinct in iron haematoxylin and is not heavily incrusted with peripheral chromatin in

nuclei in the cysts. There may be no trace of peripheral chromatin or only a layer of fine granules on its inner surface with an occasional larger granule or two in this region, but not so heavy an incrustation as in *E. coli* and *E. dysenteriae*. The intermediate zone is filled with a substance which is often much less deeply stained than the surrounding cytoplasm, but in other cases may be slightly stained in iron haematoxylin as an indistinct reticulum. We find in the nuclei of cysts no trace of inner clear halo and outer granular network in the intermediate zone such as Dobell (1920) figures for *Endamoeba coli* in some nuclei of the encysted stage.

The lightly incrusted nuclear membrane and the wide, lightly stained, intermediate zone make the nuclei of the cysts with eight nuclei stand out in the darker cytoplasm with a distinctness not equaled in other amoebic cysts of human stools. This is noticeable in preparations and is evident in some of the figures of *Councilmania* which appear in the older literature among the figures of *Endamoeba coli*, such, for example, as those of Prowazek (1911, pl. 17, fig. 18). This feature, the predominant asymmetry or lack of sphericity of the cysts, and the peculiar karyosome make possible the critical distinction between the cysts of *Councilmania* and those of *Endamoeba coli*.

There is no critical, cytological evidence from *Councilmania* which lends the least support to the hypothesis of autogamy in amoebic cysts proposed and defended by the Schaudinn-Hartmann school of protozoologists.

DIMENSIONS

The average size of the free amoebae when rounded up was 28 (20–35) microns in the case of ten measurements of amoebae from one stool. Smaller amoebae are sometimes seen which probably belong to this species. The largest free amoeba we have seen in stained preparations measured 35 by 63 microns. The amoebulae on escape from the cyst have a diameter of 7 to 8 microns. The cysts range from 8 to 34 microns in diameter. In one fresh stool in which 150 of the more spherical cysts were measured the range in longest diameter was from 11 to 34 microns, with the mode at 16 microns, the average at 16.5, and 114 cysts included between 16 and 20 microns. The curve of distribution of these measurements showed a marked skew towards the smaller diameters, and probably represents a small race of the species.

BUDDING AND ESCAPE OF AMOEBULAE

Plates 20–22, figures 15 to 28

The genus *Councilmania* appears to differ from all other intestinal amoebae of man in the occurrence in fresh stools, and therefore presumably also in the lower level, of a reproductive process of repeated budding resulting in the escape of amoebulae from the cyst. Such a process has not been observed by us in the encysted stages of *Endamoeba dysenteriae*, *E. coli*, or in *Endolimax nana*. We find no record of such a process in the cysts of any parasitic intestinal amoeba other than *Councilmania*, except possibly in *Endamoeba muris* as described by Wenyon (1907), but otherwise interpreted by him.

We have designated the process as one of budding since it may be preceded by the formation of a ridge or intracystic process which results in a protrusion of protoplasm through a small opening in the cyst wall, in the escape of a nucleus into this lobe, and in the subsequent detachment of an amoebula, to be followed by a repetition of the process until the cyst is emptied of all its nuclei.

The evidence upon which this conclusion rests is given in some detail since Dobell (1920) rejects as "an incorrect and arbitrary series of stages" the account of Mathis and Mercier (1917), who described the emergence of amoebulae from the cysts of an amoeba which they called *E. coli*, but which is undoubtedly *Councilmania*. Dobell is correct, it seems to us, in denying the existence of these phenomena in *E. coli*, but not in discrediting the *observations* of these investigators. Their observations were correct as far as they went, but they apply to *Councilmania* rather than *E. coli*. We agree with Dobell in not accepting their interpretation of a gamogenic-schizogamic cycle in *E. coli*, and find no evidence for such a double cycle in *Councilmania*.

The intracystic ridge which precedes and attends the early phases of the formation of the bud first appears as a chromophile, deeply staining tract which becomes an elevated ridge or keel of varying width which may run halfway round the cyst, or even farther, within the wall. It may be a narrow dark thread (pl. 21, fig. 19), or a blunt process (pl. 21, fig. 17), or a broader ridge (fig. 21). It is generally direct in its course, but is sometimes bifurcated at one end (pl. 21, fig. 19). In optical section it forms a distinct, elevated ridge on the contour of the cytoplasm within the cyst wall.

In a number of instances this chromophile territory seems to be definitely related to the chromatoidal mass and to proceed from one end of this structure (pl. 22, fig. 23). It appears to derive its chromophile material, which may be diffused throughout its extent, from the deeply stained substance of the chromatoidal body. Not all cysts have chromatoidal bodies and not all ridges are deeply chromophile, but they are generally more deeply stained than the cytoplasm.

The point of emergence of the cytoplasmic bud is a minute circular pore in the wall two or three microns in diameter. We are able to find this opening only in those cysts in which the bud is present. The pore is sometimes near the middle of the ridge, but is more often at one end. We find no evidence of a fixed relation of the pore to a particular part of the ridge. The diameter of the pore is less than that of the nucleus, which must therefore be elongated in passing through it. The cytoplasm in the pore is sometimes more deeply chromophile, as though condensed by the constriction (pl. 22, fig. 27).

The sequence of events in the budding process is as follows: The protoplasm protrudes through the pore (pl. 22, fig. 25), the nucleus slips out into the protoplasmic bud (pl. 21, fig. 22), the bud detaches itself as an amoebula (pl. 22, fig. 23), and a new bud forms and another nucleus creeps out. The process is repeated until the cyst is emptied of all its nuclei and as many amoebulae have escaped as there were nuclei in the cyst. We have found a few instances of residual protoplasm without nuclei left in the cyst, and no evidence of plasmotomy of amoebulae within the cyst.

The evidence for this budding process is the fact that the protoplasmic bud has been found without a nucleus, that nuclei have been seen at and near the pore, that more than one nucleus has never been seen in the bud, that the protoplasmic bud is always relatively small as compared with the original parent mass which grows smaller as the nuclei therein become fewer, that a small detached amoebula with a typical nucleus has been seen near the pore, and that in one instance a cyst with a bud in a fresh smear after an interval of an hour was found to have lost the bud. We have not as yet been able to watch the detachment of active amoebulae in the warm stage. Cytolysis of the bud and contents of the cyst was observed in one instance.

The number of amoebulae produced is eight as a rule, since there are normally eight nuclei in the matured cyst. There is, however, evidence that the number of nuclei and amoebulae may exceed this, though we have never seen cysts with sixteen nuclei. Cysts with a

bud and nine nuclei, some of them in the premitotic phase, are frequently seen. This condition suggests the occurrence at times of a fourth division resulting ultimately in sixteen cystic nuclei whose escape into the bud proceeds during mitosis, which is not synchronous in all of the eight nuclei. One cyst without a bud but having eleven nuclei has been found (pl. 20, fig. 16). In the course of our examination of preparations we have found cysts with buds and seven, six, four, three, two, and one nucleus respectively within the cyst. Continued search may be expected to reveal those with a bud and five nuclei.

The escape of the amoebulae from the cysts within the bowel is indicated by the fact that budding cysts occur in fresh, formed stools, and in liquid stools after a saline or other purge. We find no evidence that the process of budding arises or continues long in cold stools which have been kept under observation for three weeks after deposition.

Bud formation is a normal process in *Councilmania.* It appears in fresh warm stools, both with and without antecedent purge, and in patients not under drug medication. The protoplasmic ridge is not a fold of the cyst wall, but rather a protoplasmic structure. It appears in cysts in untreated smears as well as in stained slides. It can not be induced by sudden or by slow heat, by cooling, or by the action of reagents used in the preparation of slides. It is not due to trauma and can not be produced by pressure. The cysts in which it appears show no indications in nuclei or cytoplasm of abnormality.

OCCURRENCE

Councilmania lafleuri evidently has a cosmopolitan distribution similar to that of the other human intestinal protozoa. The eleven cases which we have observed in Berkeley afford no clue to the precise source of their infections. They were not contacts, and therefore presumably acquired their infections independently of one another. All but one, himself a physician, were patients under physician's care for intestinal trouble or subnormal physical conditions. Five were university students, two were from the Health Center Clinic. Six were males, one a boy, and five were females. Ten were whites and one a negress. Three were natives of California and eight were born elsewhere, of whom four had come recently to the state. One patient spent seven years in Sicily, one had lived six years in Persia, and two had

lived for some (one and ten) years in the Philippines; one was a returned soldier from France who had received since his return two treatments with emetine bismuth iodide and had been cured of an infection by *Endamoeba dysenteriae*. One patient had lived in China and in Panama, and another was born and had lived for many years in Alabama. These data present possibilities of widespread sources of infection. In addition to these cases detected in Berkeley, we have preparations of stools from several overseas soldiers in the Debarkation Hospitals in New York in 1919 containing cysts of *Councilmania*.

The cosmopolitan distribution of this infection is definitely proved by the occurrence in literature of figures of cysts of *Councilmania* attributed to *Endamoeba coli* from widely separated parts of the world. Thus Walker and Sellards (1913, pl. 1, fig. 4) figure what we regard as a cyst of *Councilmania* from Manila, P. I. Prowazek (1911, pl. 17, fig. 18) figures among his *E. williamsi* (which is *E. coli*) a cyst from Sawaii, in Samoa, which appears to be that of *Councilmania*. It is probable that some of the figures of Mathis (1913, pl. 2, figs. 2, 6, 9–14, and 16) from French Indo-China are referable here. Casagrandi and Barbagallo (1897, pl. 2, fig. 21) figure from Italy an asymmetrical cyst with bud which may be that of *Councilmania*, while Ciauri (1917, pl. 1, figs. 11–16) describes and figures an amoeba and its cysts which is undoubtedly *Councilmania* from a patient who had been with the Italian forces in Libya and Tripoli. Mathis and Mercier (1917) describe the process of budding and recognize two types of cysts in *Endamoeba coli*, but their diagrammatic figures, as drawn, appear more like *E. coli* than *Councilmania*, with the exception of figure 15. Dobell (1920) notes the resemblance of this figure to that of Mathis (1913). The sources of the amoebae discussed by these investigators are not stated. It seems probable also that James (1914, pl. 17, figs. 155–156) may have found *Councilmania* at Panama. From his account of the activities and the finding of ingested red blood corpuscles in *E. coli* it seems probable that Schiff (1919) has observed this species in troops in the German hospital at Haidar Pascha, in Turkey. This list is not exhaustive and there are other less certain suggestions of its occurrence in the literature. The data cited will suffice to indicate a wide distribution of this parasite of man comparable with though not as yet so extensive and critically founded as that of the other amoebae of man.

CULTURE AND INOCULATION

Attempts to culture *Councilmania* in diluted cat's blood and diluted human pleuritic exsudate (1 to 10) in Locke's solution have failed, as did also attempts at culture in blood clot, in blood agar (made with the blood of the cat and of the guinea pig), in plain agar, and in nutrient broth.

Attempts to culture with solid tissue under aerobic and anaerobic conditions likewise failed. We are indebted to Misses Steding and Burgis for many of these tests.

Inoculations by feeding nine young rats were negative. Cysts were discharged in the rat's faeces for not more than the first forty-eight hours, but the faeces were negative thereafter for from 33 to 77 days, and no *Councilmania* were found in six of them at autopsy. Rectal injection into four young rats has thus far given negative results. We are indebted to Mr. J. F. Kessel for these experiments.

VIABILITY OF CYSTS

In our student laboratory Mr. L. Freudenthal made a test of the viability of the cysts of *Councilmania*, using eosin staining as a test. On the 17th day 85 per cent of 233 cysts were alive, 5.7 per cent dead, and 5.3 per cent moribund. On the 31st day 85 per cent were still alive and 15 per cent dead.

Another heavily infected stool was kept at room temperature in a glass jar for forty days. The faecal matter was smeared on the sides of the container and was kept moist but not flooded. In this case there was great variation at times in the proportion of live and dead cysts observed on any given day, indicating that the cysts are not equally viable in all parts of the stool. On the fortieth day cysts were still alive in this stool with no convincing evidence that they were dying out. On the sixty-fifth day about 80 per cent of the cysts were still alive.

Cysts in human urine appear to die more quickly than in water. In five different stools the cysts were all dead in five hours in urine, but not in controls, as tested by Mr. L. Freudenthal in the student laboratory. In another instance most of them were still living at the end of forty-eight hours. The variables are so great in these tests that conclusions should be based only upon repeated experiments.

DISTINCTIONS BETWEEN *COUNCILMANIA LAFLEURI* AND *ENDAMOEBA COLI*

Since these two species both have eight-nucleated cysts the number of nuclei can not be utilized to separate the two infections as in the case of the separation of *E. dysenteriae* from the two species under discussion. There is, however, a group of characteristics which may serve in the critical diagnoses of infections by *Councilmania*. The most reliable one, the distribution of the chromatin in the nucleus, is fortunately most evident in the commonest stages, the eight-nucleate cyst. The available diagnostic characteristics are set forth below.

Councilmania lafleuri	*Endamoeba coli*
Free Stage	
Very active, pseudopodia thrust out suddenly, ectoplasm sharply separated from endoplasm.	Sluggish, ectoplasm not sharply separated from endoplasm.
Red blood corpuscles ingested readily.	Red blood corpuscles not ingested normally.
Peripheral chromatin in a thin layer, karyosome large, excentric, with halo, or often seen in premitotic condition with chromatin dispersed in granules in a sphere, ring, or skein, without halo and often central.	Peripheral chromatin in a thicker layer, karyosome small, spherical, with halo, generally excentric.
Encysted Stage	
Cyst wall very thick.	Cyst wall thin.
Spheroidal, ellipsoidal or asymmetrical, less often spherical.	Generally spherical.
Less readily stained.	More readily stained.
Glycogen body more resistant to iodine.	Glycogen body stains readily in iodine.
Nuclei with little peripheral chromatin and large, generally central or but slightly excentric, dispersed karyosome.	Nuclei with more peripheral chromatin and small, excentric, massed karyosome.
Chromatoidal bodies less acicular in early stages, fasciculate, massed centrally in later stages and contributing to chromophile buds.	Chromatoidal bodies more distinctly acicular, with less central massing and no relation to segregation of chromophile cytoplasm.
Chromophile ridge forms a bud through a pore in the cyst wall, which detaches uninucleate amoebulae.	Budding unknown.

SUMMARY

Councilmania lafleuri, gen. nov., sp. nov.

Free stage with hyaline pseudopodia, abruptly formed, endoplasm filled with food vacuoles, and even ingested red blood corpuscles, resting nucleus with moderately thin zone of peripheral chromatin, karyosome generally excentric, often with small halo, in premitotic stages composed of dispersed particles and more nearly central in location. Pseudopodia broad, rounded, usually less than diameter of the body in width, generally single. Cysts with one, two, four, or eight nuclei, rarely more, thick walled, triple contoured, spheroidal, ellipsoidal or asymmetrical, less frequently spherical. Nuclei of cysts with little peripheral chromatin and large, excentric or central, spheroidal, reniform, or lobed karyosome often divided into scattered particles, eight chromosomes, and an intradesmose joining polar masses seen best in first and second mitoses. The cysts form a chromophile ridge from which cytoplasm emerges through a pore in the cyst wall as a chromophile bud. A nucleus migrates into the bud which detaches as an amoebula. The process is repeated till the cyst is emptied of nuclei. Budding may occur in the bowel. Free stages from 63 by 35 microns to 20 to 35 microns when rounded up; spheroidal cysts 16 to 20, rarely 8 to 34, microns in diameter. Parasitic in the human intestine. Type species *Councilmania lafleuri*.

LITERATURE CITED

Casagrandi, O., and Barbagallo, P.
1894. *Entamoeba hominis* s. *Amoeba coli* (Lösch). Studio biologico e clinico. Ann. Igiene Sperim., n. s., **7**, 103–166, pl. 2.

Ciauri, R.
1917. Dissenteria amebica. Giorn. Med. Milit., **65**, 934–941, pl. 1.

Councilman, W. T., and Lafleur, H. A.
1891. Report in pathology. I. Amoebic dysentery. Johns Hopkins Hosp. Rep., **2**, 395–548, pls. 1–7.

Dobell, C.
1919. The amoebae living in man (London, John Bale, Sons and Danielsson). Pp. vi + 155, 5 pls.

James, W. M.
1914. A study of the *Entamoebae* of man in the Panama Canal Zone. Ann. Trop. Med. and Paras., **8**, 133–310, pls. 12–17.

Kofoid, C. A., Kornhauser, S. I., and Swezy, O.
1919. Structure and systematic relationships of the "Iodine Cysts" from human faeces. Military Surgeon, 45, 30–43, 25 figs. in text.

Kofoid, C. A., and Swezy, O.
1921. *Councilmania lafleuri*, a new amoeba of the human intestine. Proc. Soc. Exp. Biol. and Med., **18** (in press).

Mathis, C.
1913. Recherche des cystes d'amibes dans les selles de l'homme. Bull. Soc. Med-Chir. de l'Indo-Chine, **4**, 334–350, pls. 1–4.

Mathis, C., and Mercier, L.
1917. La schizogonie chez les entamibes intestinales de l'homme. Bull. Soc. Path. Exot., **10**, 311–322, 15 figs. in text.

Prowazek, S.
1911. Beitrag zur *Entamoeba*-Frage. Arch. f. Prot., **22**, 345–350, pl. 17, 1 fig. in text.

Schiff, F.
1919. Zur mikroskopischen Diagnose der Amöbenruhr. Arch. Schiffs-Tropenhyg., **23**, 113–127.

Walker, E. L., and Sellards, A. W.
1913. Experimental entamoebic dysentery. Philippine Jour. Sci., Ser. B, **8**, 253–331, pl. 1, 3 tables.

Werner, H.
1911. "*Entamoeba coli*," *in* Prowazek, Handbuch der pathogenen Protozoen, **1**, 67–77, pl. 2, 1 fig. in text.

EXPLANATION OF PLATES

All figures are of *Councilmania lafleuri* gen. nov., sp. nov., of drawings from preparations fixed in hot Schaudinn's fluid and stained in iron haematoxylin. The magnification in all cases is 2500 diameters.

PLATE 18

Free stages of *Councilmania lafleuri*

Fig. 1. Small free amoeba with single, broad, hyaline pseudopodium. Sharp demarcation between ectoplasm and endoplasm, very few vacuoles, and very large nucleus with central karyosome with halo, heavily decolorized. Peripheral chromatin lobed.

Fig. 2. Free amoeba with clear pseudopodium in early phase of protrusion and one in last phases of retraction filled with endoplasm at the opposite end of the body. Small nucleus with fairly heavy peripheral chromatin and large, ragged, slightly excentric karyosome with narrow halo.

Fig. 3. Free amoeba with small pseudopodium, chromophile margin at boundary of ectoplasm and endoplasm, some food vacuoles with contents, nucleus with continuous thin zone of peripheral chromatin, excentric karyosome without halo.

Fig. 4. Large free amoeba with two pseudopodia (below) in the last stages of retraction, a broad one (above) in full extension, and one recently formed (at left); endoplasm vacuolated, ectoplasm more homogeneous; nucleus with irregularly lobed peripheral chromatin, spheroidal, excentric karyosome with narrow halo.

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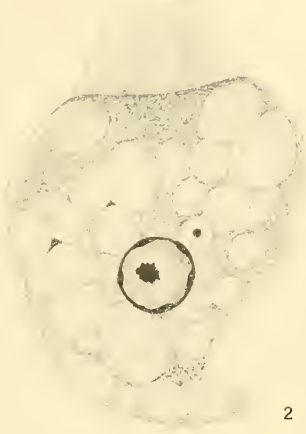

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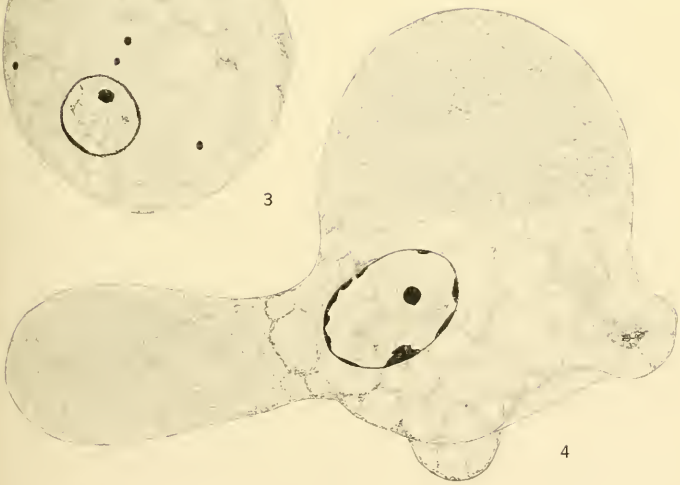

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PLATE 19

Free and precystic phases of *Councilmania lafleuri*

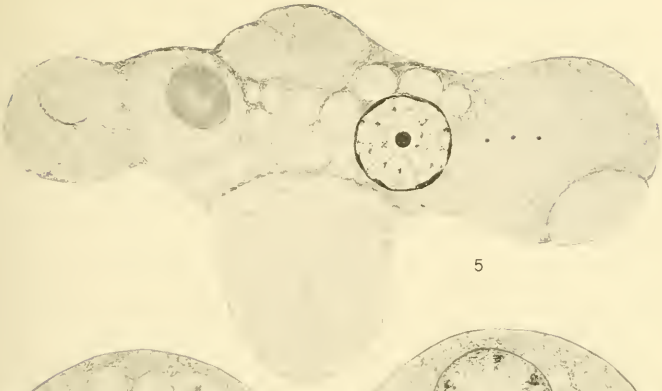
Fig. 5. Free amoeba with food vacuole containing remnant of red blood corpuscles, nucleus with a thin zone of peripheral chromatin and central, spheroidal karyosome with halo. Three pseudopodia in retraction, one at the right forming.

Fig. 6. Precystic stage with some food contents, including a cyst of *Chilomastix davainei*, in vacuoles. Halo about the nucleus, heavier zone of peripheral chromatin, very excentric karyosome with halo, intermediate zone with developing chromatin net, no ectoplasm differentiated.

Fig. 7. Precystic stage with no food contents, enlarged nucleus in premitotic phase with no peripheral chromatin zone, but with vague, destained meridional strands, karyosome slightly excentric, dispersed in a ring of granules, no well defined halo.

Fig. 8. Binucleate free amoeba with no vacuoles, and single, large pseudopodium. Nuclei with thin zone of peripheral chromatin and central, dispersed karyosome suggesting recent or coming mitosis.

Fig. 9. Rounded-up free amoeba crowded with food vacuoles containing red blood corpuscles, and bacteria, nucleus with thin zone of peripheral chromatin, slightly excentric karyosome with excentric halo.



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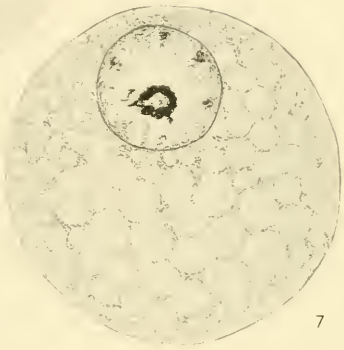

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PLATE 20

Encystment in *Councilmania lafleuri*

Fig. 10. Rounded-up free amoeba with ectoplasmic zone sharply set off from the vacuolated endoplasm. Nucleus with nearly uniform zone of peripheral chromatin, subcentral, spherical karyosome with narrow excentric halo.

Fig. 11. Very small uninucleate cyst with large central glycogen vacuole crowding the nucleus to the wall. Nucleus with neither peripheral zone nor karyosomes, its chromatin scattered in peripheral nodes with connecting strands, some of them double, a few vague chromatoidal masses in the cytoplasm, cyst wall present.

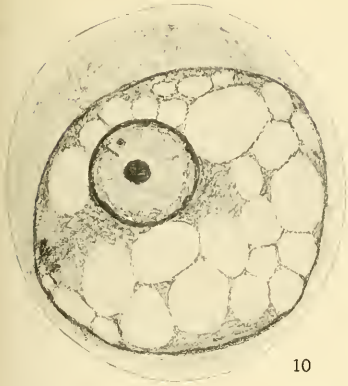
Fig. 12. Binucleate cyst with central glycogen vacuole, two nuclei in the peripheral cytoplasm nearing the prophase of mitosis, scattered flakes of chromatoidal substance in the cytoplasm, thick cyst wall present.

Fig. 13. Binucleate cyst with nuclei in early anaphase, each with sixteen chromosomes in the equatorial region and deeply stained polar masses (centrosomes) with traces of an intradesmose on the nuclear wall connecting them, small chromatoidal bodies in the cytoplasm.

Fig. 14. Quadrinucleate cyst, with cytoplasm encroaching upon the central glycogen vacuole, nuclei showing two conjoined polar masses (centrosomes) connected by strands with about eight chromatin masses on the nuclear membrane, no chromatoidal bodies, cytoplasm chromophile.

Fig. 15. Eight-nucleate, thick-walled cyst with a narrow chromophile ridge almost encircling the body, scattered chromatoidal threads traversing the cytoplasm, and a chromophile mass near the middle of the ridge. Nuclei with no peripheral chromatin, clear intermediate zone and central karyosome of dispersed granules.

Fig. 16. Eleven-nucleate cyst with central chromatoidal fascicle viewed from the end, several chromatoidal needles. Nuclei generally with subcentral dispersed karyosomes, and peripheral chromatin masses in some of the nuclei.



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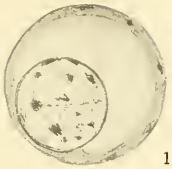

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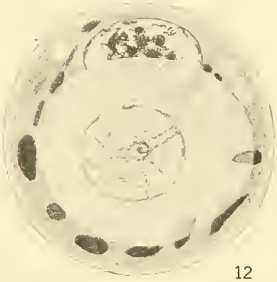

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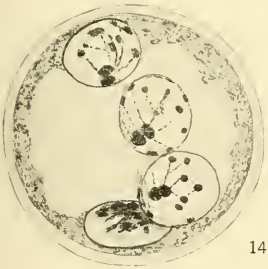

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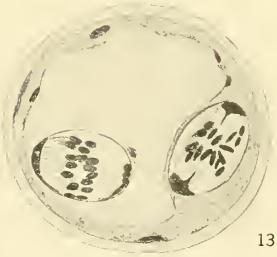

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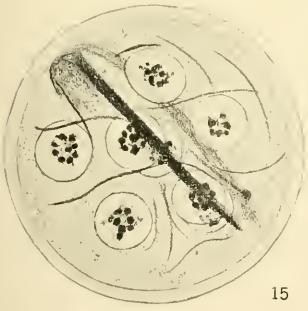

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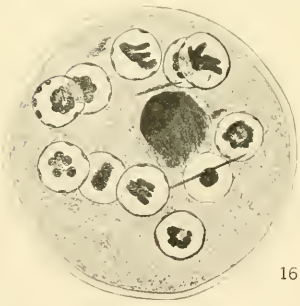

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PLATE 21

Bud formation in *Councilmania lafleuri*

Fig. 17. Eight-nucleate cyst with fasciculate chromatoidal body with one end in a bud in the early stages of formation. Nuclei with dispersed karyosomes, and some peripheral chromatin.

Fig. 18. The same stage, with chromatoidal body exhausted in formation of chromophile ridge. Nuclei with scattered chromomeres.

Fig. 19. Eight-nucleate cyst with fasciculate chromatoidal body from one end of which three narrow chromophile ridges radiate, one of which is emerging at the pore in the cyst wall at its free end. Nuclei with central karyosomes of dispersed type and little, if any, peripheral chromatin.

Fig. 20. Eight-nucleate cyst with narrow, chromophile ridge, and a bud with deeply chromophile cytoplasm emerging on top of the cyst through the pore. No nucleus in the bud. Nuclei in prophase for next mitosis. No chromatoidal body except small spherical remnant.

Fig. 21. Eight-nucleate cyst with heavy, wide, chromophile ridge, adjacent to a diffusely chromophile area of the cytoplasm, no chromatoidal body. Nuclei with central karyosomes of spireme type and no peripheral chromatin. Bud not yet formed.

Fig. 22. Eight-nucleate cyst with deeply chromophile bud containing one nucleus, nuclei of spireme type, no peripheral chromatin, small remnant of chromatoidal body left in cytoplasm.

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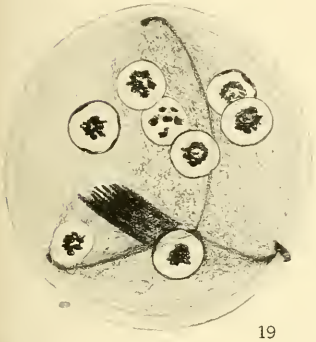

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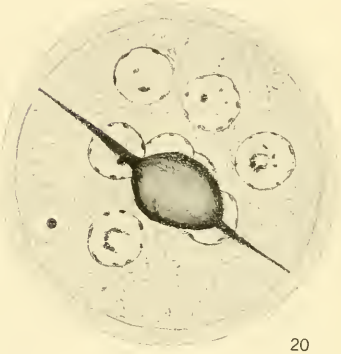

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PLATE 22

Budding in *Councilmania lafleuri*

Fig. 23. Budding cyst with central, fasciculate chromatoidal body, from one end of which a chromophile area passes out into the densely chromophile cytoplasm in the pore to the chromophile bud, or amoebula which contains a single nucleus, seven nuclei remaining in the cyst.

Fig. 24. Budding cyst in fresh smear, showing protruding cytoplasm and three nuclei remaining in the cyst. Reduction of the cytoplasm is indicated by the outline of the lobe partially filling the cyst.

Fig. 25. Cyst with small, protruding, chromophile bud, no chromatoidal body, except a nucleus-like remnant (?), three enlarged nuclei in the spireme stage remaining in the cyst.

Fig. 26. Binucleate cyst with chromophile ridge and remnant of chromatoidal body, nuclei enlarged. Pore not detected.

Fig. 27. Budding cyst with remnant of disintegrating chromatoidal body from which a faint chromophile area leads to the pore filled with deeply chromophile strand connecting with a uninucleate, deeply stained bud or amoebula. Six nuclei with peripheral chromatin in large granules remain in the cyst.

Fig. 28. Elongated cyst with no chromatoidal body but deeply chromophile area massed as though for emergence as a bud, four nuclei remaining in the cytoplasm.

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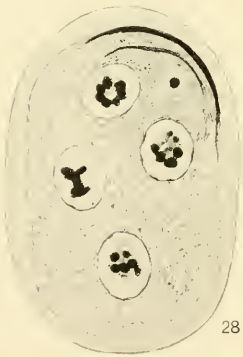

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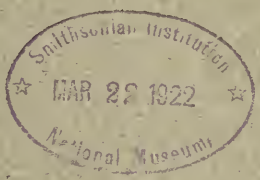
BY

CHARLES A. KOFOID AND OLIVE SWEZY



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CONTENTS

PAGE

Introduction ... 199
Material and methods ... 201
Morphology ... 202
The active flagellate ... 202
The neuromotor system and its relation to the symmetry of the organism 205
Mitosis in the active phase ... 212
The plane of fission ... 215
The origin of bilateral symmetry in the polymastigote flagellates ... 215
The encysted phase ... 217
Dedifferentiation in encystment ... 221
Function of encystment ... 222
Specific differences between *Giardia enterica* and *Giardia* from rodents ... 222
Summary ... 224
Literature cited ... 225
Explanation of plates ... 228

INTRODUCTION

Giardia enterica is a flagellate parasitic in the human intestine, and of general distribution throughout the world. It is widely prevalent in the tropics, occurs rather frequently in our southern states (Stiles, 1915), and is found more often in children than in adults. It often attends cases of acute or chronic diarrhea, achylia, duodenal ulcer, and cancer of the stomach, though clinical evidence that it is an etiological factor in these diseases is inadequate or lacking.

Species of the genus *Giardia* are found in the tadpoles (*G. agilis*) of Amphibia, in the blood(?) of the falcon (*G. sanguinis*), and in the intestine (*G. muris, G. microti, G. duodenalis, G. enterica*) of mammals, especially of rodents, cats, and man. There are morphological grounds to support the view (Bensen, 1908; Kofoid, 1920) that there are several species in the genus, and that the species in rodents differ from that found in man. This matter is important in the prevention of human infection, for if any of the species in mice and rats should be identical with that in man, the sources of infection are greatly increased because of possibilities of contamination of food supplies in the granary, warehouse, mill, bakery, and store by the infected faeces of these rodent pests. On the other hand, if the species in man is distinct from those in rodents, the sources of human infection are more circumscribed, and are limited mainly to the contamination of food or water by water-borne and fly-borne cysts from human faeces, by the dirty hand of the infected food handler, and by contaminated washbowl or towel.

It is the purpose of this paper to define accurately the morphological features of the *Giardia* parasitic in man, and thus to assist in the clearer definition of the morphological distinctions which may be used to separate this parasite from related species of *Giardia* in other mammals, notably in rodents. The accurate description will also afford a basis for the accurate microscopical diagnosis of the infection in the examination of human stools.

We have elsewhere (Kofoid, 1920) set forth the grounds under the Zoological Code of Nomenclature for using the name *Giardia enterica* for the parasite widely designated in medical treatises as *Lamblia intestinalis*. Earlier investigators (Grassi, 1881; Grassi and Schewiakoff, 1888; Metzner, 1901, among others) regarded these flagellates in man and in rabbits, rats, mice, and cats as but a single species designated as *Megastoma entericum*, and later commonly known as *Lamblia intestinalis*. In consequence of this fact figures of the *Giardia* ascribed to man have often been drawn from specimens taken from rodents and have been widely copied in textbooks as figures of the human parasites. Thus, even Fantham, Stephens, and Theobald, in their recent work on the *Animal Parasites of Man* (1916), reproduce the widely copied figures of Grassi and Schewiakoff (1888) as *Lamblia intestinalis*, although the latter authors explicitly state that their material came from rats and mice.

An added reason for the publication of a critical analysis of the morphological figures of the parasite found in man is the fact that some recently published figures in textbooks and in works specially designed for use in clinical diagnosis or in stool examination (Doflein, 1916; Cammidge, 1916; Hegner and Cort, 1921) publish or copy figures of the *Giardia* of man which give an incorrect or at least an atypical representation of the parabasal bodies, organs most useful in specific diagnosis in the genus *Giardia*. Thus Deschiens (1921) in describing the parabasals states that they are "généralement fusionnés et très polymorphes," whereas careful focusing with a good binocular microscope with immersion monobjective will almost invariably resolve the pair of parabasals accurately.

MATERIAL AND METHODS

Our material is all from human faeces and presumably belongs to a single widely distributed species parasitic only in man. It has included large and small races and infections in which ellipsoidal as well as spheroidal, or even elongated cysts were present. In the absence of other structural distinctions than those of size and proportions of the cyst, we incline to the view that these relatively rare but divergent forms are at the most but mere races within the variable species. It is quite within the range of possibilities that the species parasitic in the rodents may at times establish a foothold in man, but experimental tests of their capacity to do this and of the degree of host specificity prevalent in the genus *Giardia* are as yet lacking, except in the case of the experimental transfer of the human *Giardia* to kittens by Fantham and Porter (1916) and by Deschiens (1921).

Our material came in part from stool examinations made on 576 home service (37 cases, 6.4 per cent) and 2300 overseas soldiers (131 cases, 5.7 per cent) at the Army Laboratory, Port of Embarkation, New York City, in 1918–1919 (see Kofoid, Kornhauser, and Plate, 1919, and Kofoid, 1919), and in part from over 9000 routine stool examinations made in the Parasitological Laboratory of the California State Board of Health of residents of California, including persons from other states of the Union and from Mexico, Central America, the Hawaiian and the Philippine Islands, China, Japan, Persia, India, Siberia, and Armenia. It is thus presumably widely representative of exposures to human infections, though we have no means of establishing the actual geographical sources of the infections we have studied.

The material has been studied in fresh smears, in iodine-eosin preparations, and mainly in iron haematoxylin stain following hot Schaudinn's fluid (with 4 per cent acetic acid) as a fixer of smears upon slides, by both the slow and rapid methods of staining. Strong illumination (100 watt Mazda) and Wratten filters K1 and G and immersion oil between condenser and slide have been used in determination of the fibrillar system by the monobjective binocular microscope.

MORPHOLOGY

The fact that the investigation of *Giardia enterica* is, as a rule, dependent upon stools rather than flagellates in place in the intestine of the host, as in the case of *Giardia* in mice, puts limitations upon the amount and quality of material of the free flagellates available for investigation. The free flagellates are rare in the faeces except occasionally in diarrheic stools, and the moribund condition of many of them usually limits the material available for critical study of mitosis. It is quite otherwise in the cysts of this parasite, which find their normal conditions of life in the discharged faeces.

The Active Flagellate

The *shape of the body* is remarkably uniform in properly fixed material and is subject to but slight modifications as a result mainly of the varying degrees of contraction of the cytostome (cf. figs. 4 and 5, pl. 23, and fig. 6, pl. 24), and of the tail which may be more or less elevated (fig. D; pl. 23, figs. 3, 5) or laterally deflected (pl. 24, fig. 8). The constriction of the cytostome elevates the dorsal surface, and the tail may be elevated as much as forty-five to ninety degrees (Zabel, 1901) above the horizontal plane when the flagellate is in place on an epithelial cell of its host. In the faecal smears the tail usually lies horizontally in the plane of the peristomal region.

The body is pyriform in outline in dorsal or ventral view (fig. B), is flattened ventrally by the shallow, sucker-like cytostome, and is contracted posteriorly into the tapering tail. In lateral view, it is convex dorsally and flat ventrally, with the greatest elevation at the level of the posterior margin of the cytostome (pl. 23, fig. 2). The greatest transverse diameter of the body is at the level of the posterior third of the cytostome and slightly exceeds (0.51–0.54) half the length. The dorsoventral diameter is a little less than half the transdiameter in stained and presumably contracted material, but appears to be

relatively greater than this in living individuals. The tail is conical (25°–30°), concave laterally near the base in dorsal view, tapering to a blunt point, and is about 0.6 to 0.7 transdiameter in length. Its ventral surface is flattened and continuous with the flattened area between the posterolateral flagella. In life it is very mobile. It terminates in the posterior flagella proceeding from the axostyles.

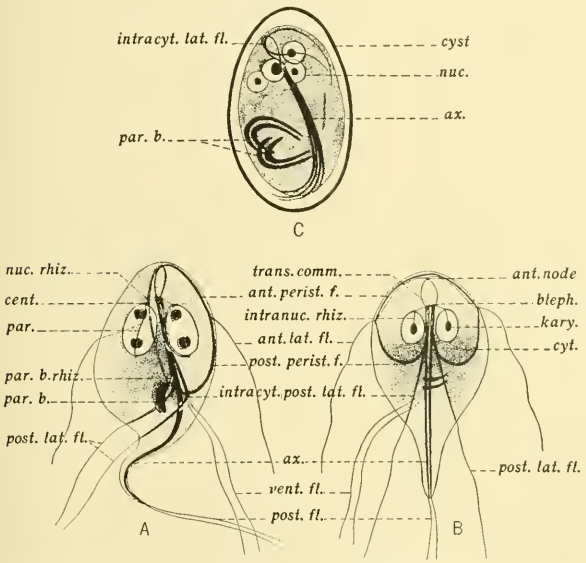
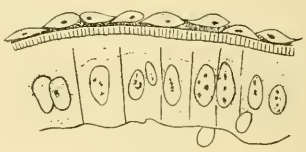


Fig. A. Lateral view of *Giardia enterica* (Grassi). Fig. B. Ventral view. Fig. C. Cyst with 2-zooid individual. Abbreviations: *ant. node*, anterior node; *ant. perist. f.*, anterior peristomal fiber; *ax.*, axostyle; *bleph.*, blepharoplast; *kary.*, karyosome; *cent.*, centrosome; *cyst.*, cyst wall; *cyt.*, cytostome; *intracyt. lat. fl.*, intracytoplasmic lateral flagella; *intracyt. post. lat. fl.*, intracytoplasmic posterolateral flagella; *intranuc. rhiz.*, intranuclear rhizoplast; *lat. fl.*, lateral flagella; *nuc.*, nucleus; *par.*, paradesmose; *par. b.*, parabasal; *par. b. rhiz.*, parabasal rhizoplast; *post. fl.*, posterior flagella; *post. perist. f.*, posterior peristomal fiber: *rhiz.*, rhizoplast; *trans. comm.*, transverse commissure; *vent. fl.*, free ventral flagella. × 2260.

The structures found in the body (figs. A and B) are two nuclei (*nuc.*), the cytostome (*cyt.*), and the neuromotor apparatus consisting of the two axostyles (*ax.*), the two parabasal bodies (*par. b.*), the peristomal fiber (*perist. f.*) around the cytostome, the centrosome (*cent.*), the rhizoplasts (*rhiz.*), blepharoplasts (*bleph.*) and the eight flagella (*fl.*).

These structures traverse the cytoplasm, which is finely and uniformly granular and exhibits no evidence of cell inclusions or food vacuoles and lacks alveolar differentiation. The peculiar anterior halo around the blepharoplasts and the triangular halo between the posterolateral flagella seen by Kofoid and Christiansen (1915) in *Giardia muris* are less sharply set off in *Giardia enterica*, in which the anterior halo appears to be little more than the thinner anterior edge of the body, and the triangular halo to show less contrast in density with the adjacent cytoplasm than in *G. muris*. This "halo" or area takes on a uniformly lighter tone as the plane of focus reaches it because of the flattening of the ventral surface between the posterolateral flagella which bound it.

D



E

Fig. D. *Giardia enterica* from vomit in case of carcinoma of the stomach. After Zabel (1901, pl. 2, fig. 41).

Fig. E. *Giardia enterica* attached to cells of the duodenum. After Müller (1888, pl. 2, fig. 2).

There are no *glycogen masses* or granules in *G. enterica*, in either the free or encysted stages. With Best's carmine (Prowazek and Werner, 1914) the cytoplasm of the cyst stains a diffuse red, indicating diffuse paraglycogen.

The *cytostome* (*cyt.*) is the concave area on the anteroventral surface encircled by the peristomal fiber. It is a sucker-like organ (pl. 23, figs. 2, 3) adapted for adhesion to the tops of the epithelial cells (figs. D, E). Müller (1890) found the flagellates on the cells of the jejunum of an executed criminal at autopsy (fig. E) and Zabel (1901) found them in place on the cells of epithelial fragments (fig. D) in the vomit from a case of cancer of the stomach. The function of the cytostome in attaching *Giardia enterica* to the cells of its host is thus proved by observation to be similar to that of *Giardia* in rodents where examination of the relations of the parasite to the cells of the intestine of the host is easily made.

The contractility of the cytostome is suggested by its variable outline in fixed material (cf. pl. 23, figs. 3, 4, 5), and by the varying ratios of the anteroposterior and transverse diameters. The postmargin is more deeply indented in the contracted state than in the uncontracted, and the anteroposterior diameter is 0.80 of the transverse in the contracted to 0.82 in uncontracted. In other words, the anteroposterior grip of the cytostome is greater than the transverse.

There is no evidence of any other function of the cytostome than that of an organ of adhesion. No gullet leads from it and no food particles can be found in the cytoplasm. We have not succeeded in cultivating *Giardia* in physiological salt solution, in culture media such as peptone broth, or in human ascitic fluid or diluted blood, in most of which the human intestinal flagellates (*Chilomastix, Trichomonas*) live for some time if frequently transferred to fresh media.

The fact that *Giardia enterica* has a powerful organ of attachment in the cytostome affords an anatomical ground for the persistence with which infection is maintained in the bowel of man when exposed to the pressure of passing food and the peristaltic movements of its substrate. In our experience it attains greater numbers than any other human intestinal protozoan.

The Neuromotor System and Its Relation to the Symmetry of the Organism

The *neuromotor system* of *Giardia enterica* is bilateral and consists of the two centrosomes and the integrated fibrillar structures attached thereto. These elements, attached to each centrosome or to the blepharoplast, are as follows: an intranuclear rhizoplast (*intranuc. rhiz.*, fig. B) passing to the central karysome (*kary.*), while peripherally a rhizoplast (*rhiz.*, fig. A) runs to the blepharoplast (*bleph.*) at the anterior end of the axostyle (*ax.*), the parabasal body (*par. b.*), the peristomal fiber (*perist. f.*), and four flagella (*fl.*). *Giardia* is binucleate and bilateral, but not diplozoic. The symmetries of the two cells and their attendant neuromotor systems stand in a reversed relation, viewed from the standpoint of other unicellular, polymastigote flagellates, notably *Chilomastix*. We find the neuromotor system duplicated as to its constituent parts, but with those of the right side arranged in the mirror image of those of the left. The constituent elements of this system are duplicated throughout, except the commissures. Joining the two systems of the right and left sides is the transverse commissure (*trans. comm.*) between the blepharoplasts,

while a second junction is effected between the intracytoplasmic parts of the anterolateral flagella in the anterior node (*ant. node*).

The *centrosome* (*cent.*, fig. A) is a minute granule at the anterior pole of the nucleus and is found with difficulty, especially in the cysts. Duplication of the neuromotor system proceeds from it through the blepharoplast at mitosis, and the paradesmose (pl. 23, fig. 7; fig. A, *par.*) is spun out between the daughter centrosomes prior to the metaphase and lies on the nuclear membrane as a dark meridional thread.

The *intranuclear* (*intranuc. rhiz.*, fig. B) and *extranuclear* (*rhiz.*) *rhizoplasts* are found with difficulty in both free and encysted stages since they destain quickly. The intranuclear rhizoplast passes to the central karyosome in the resting stage of the nucleus, and forms the line along which the longitudinal spireme forms at mitosis (pl. 23, fig. 5), as Boeck (1919) has shown in *G. microti*. The extranuclear rhizoplast passes obliquely antero-laterally to the head of the axostyle or blepharoplast.

The *blepharoplast* (*bleph.*, fig. B) in *G. enterica* is obscured more than in *G. muris* and *G. microti* by the fact that it has nearly the same diameter as the axostyle and, unless decolorization is carried on until the latter organ destains, the small blepharoplast buried in its head is not distinguishable from the contiguous axostyle. When destaining is adequate (fig. B; pl. 23, fig. 4), and especially in the prophase, the blepharoplast is seen as a small ellipsoidal body with its longer longitudinal axis buried in the head of the axostyle. To it converge all of the elements of the neuromotor system, though the parabasal rhizoplast *par. b. rhiz.*, fig. A), the ventral and posterolateral flagella, and the posterior peristomal fiber are generally so obscured by the axostyle that they cannot be followed separately to any junction with the blepharoplast. Their trend is toward the blepharoplast as far as they can be followed. From it passes the rhizoplast (*rhiz.*) to the centrosome. We have been unable to find the *two* granules figured diagrammatically by Hartmann (1910) in *G. muris* and by Rodenwaldt (1911) in [?]*G. enterica*. It is possible that they were prophetic of the prophase of mitosis (see our text-figures F–K).

The *transverse commissure* (*trans. comm.*, fig. B) is a heavy fibrous band joining the two blepharoplasts.

The four pairs of flagella (*fl.*, fig. A) all appear to arise from, or near, the blepharoplasts, in a bilateral arrangement. They all stroke posteriorly and are typically found in living and fixed material trailing more or less posteriorly.

The *anterolateral flagellum* (*ant. lat. fl.*, fig. B) passes anteriorly in a laterally convex arc to the anterior node (*ant. node*), which in an earlier paper (Kofoid and Christiansen; 1915) was called the anterior chiasma, a laterally extended granule. From this point, it is impossible in the free stage to determine whether on leaving the node the flagellum crosses over to the opposite side, or bends abruptly laterally on its own side. Either course is possible. The latter alternative seems more probable in case *Giardia* has been derived from *Chilomastix* by the morphological reversal of one daughter at mitosis (see Kofoid and Swezy, 1920). The origin of the node, however, in the prophase (figs. F to K) with the outgrowth of new anterolaterals indicates unmistakably a contact rather than a cross-over of the flagella. We therefore substitute "node" for "chiasma" to avoid the connotation of a crossing and to establish in its place one of connection only.

From this node, the flagellum runs laterally dorsal to and within the anterior peristomal fiber to near the middle of the lateral peristomal arc where it crosses that fiber, uniting with it in a slight angular prominence in its course, and emerges posterolaterally as a free flagellum. Its intracytoplasmic part stains more lightly than the adjacent, peripheral, peristomal fiber, except just before mitosis, when it is noticeably thickened and larger than the peristomal fiber. There is no distinct basal granule at or near its point of emergence from the cytoplasm in *G. enterica*. It is distinct from the peristomal fiber on careful focusing, though often confused with it by earlier investigators. The level of emergence of this flagellum appears to be characteristic in *G. enterica*, as compared with *G. muris* and *G. microti*, in that it is somewhat more anterior. In twenty-five individuals chosen at random, it was distinctly anterior to the middle in eighteen (pl. 23, fig. 1), median in five, and slightly posterior in two only. Obviously, in a mobile organ such as the sucker, the state of contraction of the peristome and the localization of the contraction modify the relative positions of the emergence of this flagellum.

The *posterolateral flagella* (*post. lat. fl.*, fig. B) emerge posterolaterally at the level of the constriction of the tail. Their points of emergence are about the same distance from those of the anterolaterals as the latter are from the apex of the body. Their intracytoplasmic parts (*intracyt. post. lat. fl.*) pursue an anteromesad course, apparently uniting with the posterior peristomal fibers as they curve anteriorly and merge with the axostyles on their way to the blepharoplasts. Presumably they join the blepharoplasts of their respective

sides. We have been unable to detect them as separate fibers parallel to the axostyles as in Hartmann's (1910) diagrammatic figure of *G. muris*. The intracytoplasmic sections destain with difficulty, and, next to the peristome and axostyles, are the heaviest fibrillar parts of the neuromotor system. There is no basal granule at or near the point of emergence of these flagella. Their position indicates that they are possibly the homologues of the flagellum of the undulating membrane in trichomonad flagellates.

The free *ventral flagella* (*vent. fl.*, fig. A) emerge close together at the level of the posterior margin of the cystostome (pl. 23, figs. 2, 3) and appear to merge with the axostyles at about their level. We are not able to trace them forward as distinct fibers to their respective blepharoplasts. In life, these flagella are extraordinarily active, moving in unison, and in fixed material usually maintain their parallelism.

The *posterior flagella* (*post. fl.*, fig. A) are free extensions of the axostyles emerging at the tip of the tail and are without basal granules.

The *axostyles* (*ax.*, fig. B) are two tapering, closely parallel rods of deeply staining or grayish fibrillar substance passing posteriorly from the two blepharoplasts. They and the adjacent parabasals constitute the most striking elements of the neuromotor system in the free stages, and especially in the cysts in the process of multiple fission. The blepharoplasts are lodged in the anterior ends of the axostyles and are not distinguishable from the axostyle except by adequate destaining. The axostyles are continued distally as the free posterior flagella and are thus morphologically the intracytoplasmic parts of such flagella. The lively movements of the tail in whose ventral surface they lie demonstrate the motor function of these axial organs.

The question as to the homology of this organ of the component cells of *Giardia* to the axostyles of other polymastigote flagellates is an interesting one. We regard it as homologous with the slender rod in *Chilomastix davainei* which we (Kofoid and Swezy, 1920) called the parastyle. In *Chilomastix*, this organ lies in a position with regard to other organs, notably the cytostome and blepharoplast, homologous to that of the axostyle in the right cell of *Giardia*, and in the left cell to that which it would occupy in the mirror image of *Chilomastix*. It does not, however, reach the posterior end of *Chilomastix* nor form a free flagellum at its tip. It differs from the axostyle of *Trichomonas* and related genera in the development of the free

flagellum at its tip and in being more condensed and more deeply stainable, though not in contractility and its general morphological relations.

It is to be expected, in so diverse a group as the polymastigote flagellates, that there will be a wide range of form, development, and possibly of modifications of function, in so fundamental an organ as the axostyle, that these diversities will increase as our knowledge of the group is extended, and that the evidence for the homology of the diverse types will be strengthened as more types come to light.

The *parabasal bodies* (*par. b.*, fig. A) are a pair of comma-shaped structures lying in the cytoplasm on the dorsal side, just posterior to the middle of the body and to the posterior peristomal fiber.

Their position in the cytoplasm is in sharp contrast to that of all other intracytoplasmic structures which trend more or less posteriorly. The parabasals, on the other hand, generally lie transversely or obliquely to the major axis with their larger blunter ends near the axostyles and the tapering pointed ends directed laterally, indifferently to right or left or obliquely dorsally, and reaching, in ventral or lateral view respectively, about half the distance to the periphery. Their length in the free stage is equal to or a trifle more than that of the longer axis of the nucleus. They are curved bodies, shaped like a comma without a head, and taper from a bluntly rounded, larger end directed mesially, to a finely pointed end directed peripherally. From their blunter ends a fine thread, the parabasal rhizoplast (pl. 24, fig. 9; *par. b. rhiz.*, fig. A), runs anteriorly into the region of the axostyle, and presumably thence to the blepharoplast. Rodenwaldt (1912) incorrectly denies that these bodies have any relation to the fibrillar system. The individuals so oriented as to exhibit the parabasal rhizoplast are rare, and long searching of abundant material has been necessary to detect this connection.

The most striking and persistent feature of the parabasals is their uniform parallelism. This appears in both the free and encysted stages and in mitosis when daughter parabasals are formed by longitudinal splitting. It is an expression of the bilaterality of the organism which manifests itself in the mutual relations of the elements of this pair of organelles, though the pair itself may lie in a great variety of positions in the body, in but few of which do its members exhibit the definite right-left position of the other organs.

The parabasals stain uniformly in varying tones of black in iron-haematoxylin and resist destaining quite as much as the karyosome,

except at mitosis, when they tend to destain. They are the most prominent organelles in the living and in the stained cyst because of their optical properties and stainability.

The parabasal bodies tend to fade out at mitosis in the free flagellates (pl. 24, figs. 7, 8, 11) and are found at the metaphase only with the greatest difficulty, if at all, often only as vague clouds in heavily destained slides. They are not homologues of the chromatoidal bodies of intestinal amoebic cysts, but their enlargement during encystment and their dense stainability during mitosis in the cysts of *Giardia* strongly suggest their functional analogy to the ephemeral chromatoidal bodies of the cysts of the intestinal amoebae.

The homology of the parabasals of *Giardia* is indicated by their shape which is that of a curved rod; by their position with relation to the posterolateral flagella, the homologues of the undulating membrane of the trichomonads; by their connections by a rhizoplast with the blepharoplasts; and by their behavior in mitosis. They are homologous with the curved parabasal of *Chilomastix* (parabasal, Kofoid and Swezy, 1920) alongside the cytostome with its undulating membrane; with the curved chromophile rod in the base of the undulating membrane of *Protrichomonas, Tritrichomonas, Trichomonas, Pentatritrichomonas* and *Trichomitus,* which we have called (Kofoid and Swezy, 1915, 1920) the parabasal; and with the chromophile rod coiled about the axostyle in *Parajoenia* (Janicki, 1911). There are two of them present in *Giardia* because this genus is a bilateral, binucleate organism derived from the trichomonads by duplication of the nucleus and its attendant neuromotor system, including the parabasal, and by a reversal of symmetry of the neuromotor system of one of the two constituent cells.

It is to be noted in this connection that the two parabasals lie asymmetrically in the cytoplasm and that the sagittal plane which parts them does not coincide in vertical position with that parting the other organs. In other words, the morphological sagittal plane which passes between the two parabasals is variously inclined in the dorsal protoplasmic mass according to the varying positions of this pair of organs. It is characteristic of the parabasal of some polymastigote flagellates to vary considerably in its position, as for example, in *Parajoenia,* although it is fairly constant in its relation to the undulating membrane in the trichomonads.

The parabasals of *Giardia enterica* present a great variety of appearances according to their position and their relations to stages of

mitosis. Typically they have the form above described, but in some aspects, unless carefully focused out, they appear as a *single* mass. For this reason, and also because in a moribund state degenerative phenomena affect these organs, they have been figured as a single median structure, a misleading and certainly atypical condition. Thus Benson's (1908, fig. 2, see also Doflein, 1916, fig. 642*b*) figure of *Lamblia intestinalis* from man has a single stout clavate parabasal which probably is only the superposed members of the pair of parabasals. The same type of parabasal appears in Hegner and Cort (1921, pl. 4, fig. 6) in Simon's figure of *Giardia intestinalis* from man. The single parabasal appears in Grassi's (1883, pl. 3, figs. 3, 4) original figures of *Megastoma entericum* (host not stated but presumably man) and in those of Grassi and Schewiakoff (1888, pl. 15, fig.8) of *Megastoma entericum* from rodents as well as in some of Metzner's (1901, pl. 15) figures of *Megastoma entericum* from the rabbit. From the nature of the figures it appears that failure to focus out and analyze the pair of parallel parabasals underlies the interpretation or figure of these organs as a *single* rather than a paired structure. We find it possible to analyze the seemingly single bodies into the pair in our material as a general rule and regard the other interpretations as erroneous.

The *peristomal fiber* (*ant.* and *post. perist. f.*, fig. B) is a deeply staining thread encircling the so-called cytostome of *Giardia*. It lies in the immediate periphery of the concave depression on the anteroventral surface of the body. Its anterior half has an almost semicircular course, while posteriorly it has a recessed reniform one, the lateral arcs incurving anteriorly to the median line, along the inner ends of the posterolateral flagella. The incurved arcs appear to fuse with anterior sections of the axostyles as the latter curve dorsally over the recessed cytostome. Along the inner edge of the anterolateral portion runs the intracytoplasmic section of the anterolateral flagellum closely parallel to and often obscured by it. The two are often drawn as a single fiber, as in the figures of Prowazek and Werner (1914) and of Simon in Hegner and Cort (1921). At the point where the flagellum crosses over the peristomal fiber to emerge from the cytoplasm, often slightly posterior to the middle of the lateral arc, there is generally a slight angle in the otherwise regular curvature of the arc, but we have found no granule there, as in *Giardia microti* and *G. muris* (Kofoid and Christiansen, 1915), though one appears in Simon's figure.

The peristomal fiber is continuous across the median line in the anterior arc, though at that point in some individuals it may be reduced somewhat in size and degree of stainability.

The peristomal fiber of *Giardia* is the homologue of two peristomal fibers of the trichomonads. Its dual origin is not shown on the anterior margin but is preserved in the posterior one. The right half is an identical homologue of the well-developed, sucker-like peristome of *Chilomastix* and *Waskia* and of the feebly developed one of *Trichomonas, Tritrichomonas,* and *Pentatrichomonas.* The left half is a reversed homologue, being a mirror image of the right. As in *Chilomastix* the right half lies at the right of the axostyle and near a curved parabasal, and a blepharoplast lies to its left.

The function of the peristomal fiber is unknown. It lies in the margin of a mobile organ of adhesion in the part in closest contact with the cells of the host, and in an area of maximum mobility in contraction in the adhesive functioning of the cytostome. In so far as its morphological relations indicate its function, it may be either contractile or conductile (sensory?) or possibly both in a primitive undifferentiated state.

We have thus seen that the fibrillar system joining karysome, centrosome, blepharoplast, cytostome, parabasal, flagella, and axostyle, and connected across the median plane by the anterior node, transverse commissure, and anterior peristomal fiber, forms a structurally integrated whole of the organs concerned in locomotion and adhesion. We will now consider their relations in mitosis.

Mitosis in the Active Phase

Owing to the fact that active flagellates are discharged in the faeces under unusual conditions in the bowel from the site of the infection at some distance up the intestine where multiplication by binary fission is presumed to take place normally, the normal condition of the chromatin is subject to modifications due to the moribund state of the flagellates. The central karyosome is usually rounded up and mitotic figures are generally rare in the free stages obtained from stools.

The following facts are established by available data. First, mitosis, and presumably its culmination in binary fission, takes place in the free stage in the bowel (pl. 23, figs. 4, 5). Secondly, there are four chromosomes, appearing as rounded-up spheroidal masses (pls. 23 and

24, figs. 4–12). Thirdly, the neuromotor system is duplicated at the time of mitosis (pl. 24, figs. 10, 11). The method of duplication is less certain. This appears to take place by duplication of the centrosomes and blepharoplasts by division, by the longitudinal division of the axostyle and parabasals, the former from the anterior end posteriorly, and by the splitting of the peristomal fiber from the posteromedian end anteriorly.

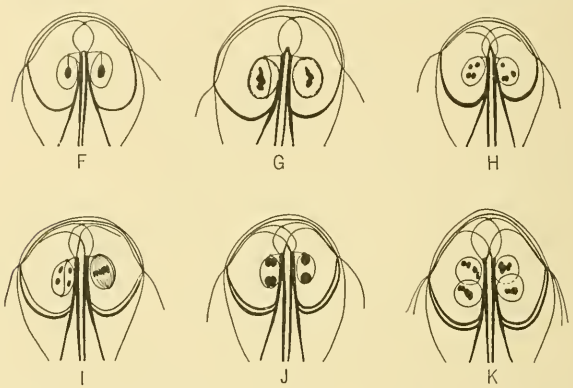
The question as to whether these organs split or are duplicated by distal outgrowth of a new element whose growing distal end alongside the old lies near or in contact with the older structure, is largely one of interpretation. In the absence of free ends of new outgrowths and of young or small axostyles and parabasals, the morphological evidence favors the interpretation of splitting for these organs, though either is possible. The equality of size and peripheral contact of the two elements support the interpretation of splitting.

There is, however, clear evidence in support of the view that the flagella are duplicated by the outgrowth of one new set rather than by splitting of the old. There is a new outgrowth of a pair of anterolaterals at the prophase shortly after the division of the centrosome and formation of the paradesmose (pl. 24, figs. 7–10, and text figs. F–K). The sprouting flagella when first seen (fig. F) form an inverted V-shaped, anterior projection from the blepharoplasts, the apex of which is the anterior node. It appears to involve the whole of the transverse commissure. This structure is figured by Simon (see Hegner and Cort, 1921) but not interpreted as here stated. It is typical of the prophase only. The anterior outgrowth continues until the node is carried out near the parental one (fig. H) and the growing flagella extend from it beyond the node in the laterally arched processes which come ultimately to be dorsal to the older flagella. At no time until the completion of the anterior progression of the node is there even the semblance of origin by splitting. It is clearly a case of origin by outgrowth of one new flagellum duplicating the parental one which persists. The homologous flagellum of *Chilomastix* and the trichomonads also arises by new outgrowth.

There is a suggestion of a similar sprouting process in the ventral and posterolateral flagella in the fact that at the prophase there appear deeply stained enlargements of the intracytoplasmic parts of the posterolaterals near their junctions with the axostyles and on the axostyles at the points where the ventrals appear to join them. These enlargements we interpret as local growth phenomena preparatory to

the outgrowth of new flagella. It is probable that "basal granules" occasionally seen at the points of emergence of the flagella are prophase phenomena suggestive of the emergence of new flagella.

Rodenwaldt (1912, fig. 4) in Prowazek, *Handbuch der Pathogenen Protozoen,* has given an entirely different, and in the light of our evidence, an incorrect interpretation of the nature of this outgrowth. His figures 4-I to 4-V fail to show the progressive changes in the



Figs. F–K. Several stages in the origin of the new anterolateral flagella from the blepharoplasts and transverse commissure by new outgrowth establishing the basis for the fission of the organism in the longitudinal frontal plane. × 2400.

Fig. F. Slight anterior projection of the transverse commissure. Karyosome elongating in nuclear axis along the intranuclear rhizoplast.

Fig. G. Transverse commissure carried anteriorly in the form of an inverted V. Paradesmose formed, karyosome transforming into longitudinal skein.

Fig. H. New anterolateral flagella diverging from the tip of outgrowth. Skein resolved into chromosomes.

Fig. I. Continued outgrowth of anterolateral flagella. Posterior peristomal fiber divided. Nuclei approaching metaphase.

Fig. J. Outgrowth of anterolaterals reaches the periphery. Nuclei in anaphase.

Fig. K. Outgrowth of free ends of new anterolaterals. Mitosis completed.

V-shaped process and he incorrectly connects the axostyles with the tip of the process, whereas they retain their connections with the blepharoplasts from which the parental anterolateral flagellar arch springs. His figures also incorrectly leave these blepharoplasts stranded on the rhizoplasts. Wenyon and O'Connor (1917, pl. 2, figs. 1–5) figure correctly but do not discuss the significance of several stages in the origin of the daughter anterolaterals. They separate the

two blepharoplasts at the head of each axostyle; in our material the destaining was not carried far enough to detect the separate granules, if such exist.

The Plane of Fission

The plane of fission (pl. 24, fig. 12) is a longitudinal frontal one rather than a sagittal one. In consequence of this the parent nuclei of the right and left sides and their attendant neuromotor systems give rise by division or outgrowth to homologous parts of the daughters, whereas if the organs of the two sides each gave rise to a daughter, each lateral half of the neuromotor system must then necessarily give rise to its supplemental half by a reversal of symmetry of the added half, that is, the nucleus and neuromotor system of the right side would at mitosis have one daughter cell retaining the parental symmetry and the other assuming its mirror image. The telophase figured (pl. 24, fig. 8) shows clearly that this does not take place, but rather that the symmetry of the parental nucleus-neuromotor system of the whole organism is reproduced in the daughters by the frontal division.

The *paradesmose* arises between the daughter centrosomes in the prophase. The centrosome divides and one daughter migrates on the outside of the nuclear membrane to the posterior pole of the nucleus, spinning out a deeply staining strand, the paradesmose, between the daughter centrosomes. This has no constant position, can be found only when favorably located for observation (pl. 24, fig. 7), and soon fades out as the metaphase passes.

The Origin of Bilateral Symmetry in the Polymastigote Flagellates

The polymastigote flagellates present two unique types of multicellular somatellas, distinct in organization from the dendritic (*Dinobryon*), and radial (*Synura*) types evolved in the lower orders of the Euflagellata, and from the geometrical types of plate (*Gonium*), ellipsoid (*Pandorina*), sphere (*Volvox*), and flattened, spirally bilateral and anteroposteriorly differentiated ellipsoid (*Platydorina*), evolved among the Volvocidea.

These two unique types of somatellas are alike anteroposteriorly differentiated in the location both of nuclei and of their attendant neuromotor systems; but in the number of nuclei and in their grouping and in the interrelations of their attendant neuromotor systems, they present two distinct types of symmetry, the spiral and bilateral.

The spiral type is represented by *Stephanonympha* and probably by *Calonympha,* multicellular parasites of the termites, in which the nucleus-neuromotor units are spirally grouped in the anterior end of the asymmetrical body of the organism. The direction of the spiral is unfortunately not clearly established in Janicki's (1911) figures. It is obvious that this type of organismal symmetry is dominated by the spiral organization typical of the body of the unicellular flagellates. There is, in so far as is known, no fibrillar connection between the cellular units of these somatellas beyond that achieved by the proximity of axostyles.

The bilateral type found in the Hexamitidae is, on the other hand, a very great morphological departure from the fundamental primitive, spiral, organismal organization so widely expressed among the Mastigophora. It involves two fundamental departures from the organization of these spiral somatellas. The first is the existence of the transverse commissure and its derivative, the anterior node, which unite the cells of the right and left sides of the body. The second is the reversal of symmetry of the nucleus and neuromotor system of the one side so that it constitutes a mirror image of these organs of the other side. Given the spirally asymmetrical, uninucleate flagellate, representing the right half of *Giardia,* this morphological reversal affords the only method by which a binucleate, bilateral organism (*Giardia*) might have been evolved from the uninucleate spiral one (*Chilomastix*). We do not mean to postulate that *Giardia* itself was evolved from *Chilomastix,* since these two genera are highly differentiated representations of their respective groups, but assume that the divergence of the binucleate group occurred in some more primitive condition of ancestral representatives.

The morphological contrasts involved in the evolution of sinistral and dextral, spiral uninucleate flagellates, as illustrated in certain parasitic flagellates of the termites, such as *Dinenympha gracilis* Leidy and *Personympha flagellata* Grassi, are comparable in type with those reversals in stereometric relations which exist between organic compounds such as laevulose and dextrose and between sinistral and dextral albuminoids. Should organ-forming substances be in the control of symmetry in flagellates, their potency in establishing the sinistral and dextral types of organisms is to be sought in their own sinistral and dextral molecular structure.

The morphological modifications of symmetry involved in the evolution of the Hexamitidae from the Trichomonadidae, or of *Giardia* from

Chilomastix, involve not only the origin of reversed symmetry, but the additional factor of the union in one individual, not of two individuals of *Chilomastix*, but of *Chilomastix* and its mirror image. The fibrillar union of the neuromotor systems of the two into one integrated system perfects their functional coördination in one organism.

Since the bilateral Hexamitidae combine in one organism the sinistrally and dextrally organized halves, we may infer (Kofoid and Swezy, 1920) that the sinistral half might have arisen in evolution by a reversal of symmetry at one pole of the nucleus at mitosis, resulting in one daughter organism being the mirror image of the other, a relation permitting union of the two in a single bilateral organism. That such a reversal is dependent upon a stereometric reversal of an organ-forming substance is an inference inviting further investigation on other than morphological lines.

The Encysted Phase

Cysts of *Giardia enterica* are abundant at irregular intervals in the stools of infected persons. In the intervals between the periods of abundance they decline in numbers or disappear entirely for periods of several days to several weeks. The numbers in which they may appear are large. Porter (1916) reports a case in which a stool of 950 cc. contained, on computation, 14,440,000,000 cysts or 15,200,000 per cubic centimeter. In a case of giardiasis under daily examination by us in New York for forty-two days, cysts were found on haemocytometer count on seventeen of the forty-two with a maximum record of 3,925,000,000 *Giardia* in a stool of 365 grams, or 21,216,216 per gram.

The cysts are found in all stages of multiple mitosis, that of the single zooid similar to the free stage (pl. 24, fig. 13) up to the 12- and presumably the 16-nucleate, 8-zooid stage (pl. 26, fig. 31). The single zooid and the 8-zooid stages are, however, very rare in our material. The period of encystment in the bowel is one of multiple fission, but apparently not of plasmotomy. The presence of a considerable proportion of 2-zooid cysts in the faeces suggests the possibility that some cysts do not proceed beyond binary fission. On the completion of plasmotomy such cysts might give rise to so-called copulation cysts with two individuals in end-to-end, back-to-back position.

Multiple fission gives rise successively to 2-, 4-, and 8-zooid stages with 4, 8, and 16 nuclei respectively, and attendant, but often lagging neuromotor systems lacking the free flagella, with cytoplasm as yet

undivided. The pairs of curved parabasals are especially prominent and paired axostyles are evident, so that the cyst appears to have a tangle of overlapping, stained strands from which the constituent pairs of nuclei and their attendant neuromotor systems are disentangled with increasing difficulty as the divisions progress within the as yet undivided cytoplasm. The increased size of the deeply staining parabasals, the curvature of the axostyles in the confinement of the cyst, and the shifting of the nuclear pairs as mitosis proceeds, add to the variety of relations presented in the successive stages of multiple mitosis (pls. 25 and 26).

The cysts are ellipsoidal bodies of hyaline, bluish, highly refractive optical properties. They are quickly and easily discerned among the faecal débris and are at once distinguished from all other cysts in the faeces by their ellipsoidal form and the prominent parabasals and axostyles which, in the fresh state, in Lugol's solution, in iodine-eosin, and in iron haemotoxylin stains, stand out even more clearly than do the nuclei.

The shape of the cysts varies considerably in different races. Figures on plates 25 and 26 were chosen to represent these variations as well as those of a fluctuating nature. The most abundant race is that represented in the ellipsoidal rather than spheroidal or elongated cysts. These are symmetrically ellipsoidal, with the major axis 1.3 to 1.7 times the transverse in length. In some cysts (pl. 25, fig. 18) the anterior end tapers slightly, giving an ovoidal form to the cyst, thus reversing the proportions of the free stage. There is a stout race, the free stage of which is as yet not studied, which has a cyst approaching the spheroidal form with an axial ratio of 1 to 1.11 to 1.22 (pl. 25, fig. 16; pl. 26, fig. 23). This is not based on end views of ellipsoidal cysts.

This stout race was found together with the ellipsoidal in one instance of infection but it was the sole or predominant one in several others, supporting the inference that this type of cyst may represent a form-size race. The elongated type (pl. 25, fig. 21; pl. 26, fig. 24) is probably not a form-race, but occurs in small numbers among the ellipsoid cysts. In some instances in stained cysts the cytoplasm has withdrawn locally from the cyst wall as though shrunken by artefact. These lacunae also appear in some cysts in fresh smears as though the cyst might under some circumstances contain more space than the volume of the protoplasmic body. A few cysts (pl. 25, fig. 19; pl. 26, fig. 30) are irregular in outline.

The size of the cysts varies from 8.5 by 11.3 to 11 by 19μ. Spheroidal races are 11.1 by 11.7 to 10.8 by 13.4μ.

The cyst wall is a hyaline, homogeneous structure of uniform thickness throughout, less than 0.5μ in thickness. Its formation evidently begins as a secretion about the as yet uncontracted 1-zooid body. In figure 13, plate 24, an individual is shown in which the cyst is in the process of formation, while the tail is not, as yet, fully retracted within the ellipsoidal mass characteristic of the encysted phase, and the cyst wall shows two separated layers posteriorly, the inner one of which has the contour of the free stage.

The following structures only can be identified as taking part at some stage at least in multiple mitosis: the nuclei, centrosomes, blepharoplasts, parabasals, axostyles, intracytoplasmic parts of the posterolateral and the anterolateral flagella including the anterior node. Free flagella and the peristomal fiber disappear and the rhizoplasts are found with difficulty, if at all.

The sequence of events within the cyst is as follows: Encystment of the 2-nucleate, 1-zooid flagellate occurs within the bowel, presumably at or near the locus of infection. Since cysts are discharged in fresh stools in various stages of multiple fission, it is certain that successive mitoses proceed as the cysts are in transit through the bowel, but that the rate and stage attained vary among the cysts. Many of them are in the 4-nucleate, 2-zooid and 8-nucleate, 4-zooid stages, few are discharged in the 2-nucleate, 1-zooid, and 16-nucleate, 8-zooid stages.

The individuality of the zooids formed by mitosis in the period prior to plasmotomy, which is so marked in the struggling zooids of the 8-celled somatellas of *Trichomonas* (Kofoid and Swezy, 1915), is manifested in *Giardia* at the close of the first mitosis when the two pairs of nuclei and their attendant neuromotor systems may, in some cysts, shift to opposite poles of the cyst (pl. 25, fig. 21; pl. 26, figs. 24, 30). That they do not always attain or retain this position is indicated by the occurrence of 4- and 8-nucleate cysts with the nuclei all at one end (pl. 26, figs. 23, 28). This variability in position suggests some mobility and functional and structural individuality of the constituent zooids of the cysts of the as yet common cytoplasmic mass. In other words, the individuality of the binucleate zooids is maintained throughout the life of the cyst and the common cytoplasm appears to be merely the containing medium. The cyst of *Giardia* is not comparable with a cleaving egg but rather with a multiple cysticercus or chain of planarians. It is asexual reproduction of the *Giardia* zooid which progresses in the cyst.

Plasmotomy does not often occur within the cysts, in so far as we have observed them, though seen in *G. muris*, and duplications of the

neuromotor system or parts of it often lag behind nuclear mitosis. The duplication of parabasals (pl. 26, fig. 24) proceeds most nearly at the same rate with that of the nuclei, though it may be accelerated or delayed, the axostyles and posterolateral flagella follow closely thereafter, and the other elements are less active. There is no constant uniformity in the relative rates of the nuclei (pl. 25, fig. 22), some, singly or in pairs, dividing before others. Likewise the axostyles may remain undivided (pl. 26, fig. 31) while the nuclei have proceeded to the 8 (at one end)–16 (at the other end) cell stage. Since the rate at which cysts of *Giardia* die varies among cysts of the same and of different stools, this variability in fission rate of different organelles is an index not only of viability but of the extent of the pathological conditions present among the cysts. Abnormal rates and irregularities of mitotic and fission phenomena are thus indications of pathological conditions in *Giardia* which are comparable with pathological irregularities in cleavage and in fission in the metazoan individual.

Nuclear conditions in the cysts throw some light on the nature of mitosis in *Giardia*. The chromatin elongates in the axis of the nucleus along the intranuclear rhizoplast (pl. 26, fig. 25), as in *G. muris* (Boeck, 1916). Prior to the metaphase the four chromosomes assume a parallel and later, in the equatorial plate, an end-to-end position (pl. 26, fig. 26). There is also an increase in the amount of granular, peripheral chromatin on the nuclear membrane in the prophase (pl. 23, fig. 5) and a rounding up of the nucleus. The volume of the nuclei decreases somewhat as the mitoses progress, but varies considerably during the different phases of the process. The nuclear membrane remains intact at all times. A paradesmose joining daughter nuclei is sometimes seen (pl. 26, fig. 30).

One of the most remarkable features of the encysted stage is the very considerable increase in mass, 100 to 400 per cent, of the parabasal bodies. In this regard they behave in close analogy with the chromatoidal bodies of the amoebic cysts of the human intestine. In these cysts the chromatoidal bodies develop as the glycogen mass decreases and disappear after mitoses have been completed. There is no glycogen body in *Giardia* but the parabasals increase greatly during the first two mitoses and seem to be reduced somewhat in the older cysts (pl. 26, figs. 30, 31). There are occasionally small chromatoidal granules (pl. 25, fig. 17) lying free in the cytoplasm, but they are ephemeral and rare. They may in some instances represent remnants of the peristomal fiber.

Dedifferentiation in Encystment

During the encysted period those parts of the organism functioning in locomotion and attachment dedifferentiate, namely, the free ends of the four pairs of flagella, the sucker-shaped cytostome, and peristomal fiber, all of which disappear promptly when the body rounds up within the cyst wall, except the posterior peristomal fiber, which lingers for a time. A similar dedifferentiation does not occur in the mitosis of the free stage. Even the newly formed intracytoplasmic parts of the anterolateral flagella stain feebly and lag in formation in the cyst. Similar parts of the posterolaterals, however, stain heavily and are more evident. A similar phenomenon appears in somatellas of *Trichomonas*, in which the homologue of the posterolateral of *Giardia*, the marginal fiber of the undulating membrane, is deeply stained during multiple fission.

The posterior peristomal fiber shifts in location with the disappearance of the cytostome and the anterior peristomal fiber. Its median end moves posteriorly and the two sides form a V-shaped structure (pl. 25, fig. 18) which gradually fades out. In this condition the peristomal fiber tends towards the more elongated condition seen in *Trichomonas* and *Chilomastix*. The connection of this V-shaped structure with the anterior part of the peristomal fiber persists for a time (pl. 25, fig. 22), but the former soon disappears and only the latter can be found. It is not to be confused with persistent intracytoplasmic parts of the posterolaterals or the parabasals.

On the other hand, the axostyles and parabasals are enlarged and stain more deeply in the cysts than in the free stages and their duplication proceeds in step with the mitoses. The axostyles are important organelles in the separation of the zooids at plasmotomy of the somatella, and the parabasals appear to be concerned with metabolism, perhaps in a fashion analogous to that of the chromatoidal bodies in amoebic cysts in stools, and are therefore not dedifferentiated as are the functionless, free flagella.

Function of Encystment

The encystment of *Giardia* has the same dual functions as in that of the intestinal amoebae. It provides, in the first place, a resistant stage discharged in the faeces of the infected host, which survives under favorable conditions of temperature and moisture and may serve to infect a new host by the oral route. It serves a second function in providing for the period of multiple fission of the encysted *Giardia* as Wenyon (1915) proposed. It results in the formation in the common cytoplasm of the organs of 2, 4, and 8 individuals which presumably separate at encystment. We find no evidence whatever to support the opinion of Schaudinn (1903, p. 550) and later defended by Penfold, Woodcock, and Drew (1916), that there are copulation cysts in *Giardia*, or that of Hartmann (1910, p. 47) that autogamy occurs in the cysts, and that there is an *Octomitus* stage. It is to be noted that Hartmann and Schilling (1917, p. 169) admit the uncertainty of sexual processes in the cysts. We find neither maturation, fertilization, sexual behavior, nor zygotes. We regard the so-called copulation cysts as cysts in which binary fission and plasmotomy are completed and the two individuals are sister zooids, separated and awaiting escape, not gametes to fuse into a zygote. We have not found such binary cysts in *G. enterica* from man, though we did find them (Kofoid and Christiansen, 1916) among the cysts of *G. microti* and *G. muris*. Furthermore, the opinion hazarded by Penfold *et al.*, that only a single individual will be liberated from the cyst of *Giardia* finds no support in our analysis of multiple fission in the cyst.

Specific Differences between G. enterica and Giardia from Rodents

Giardia enterica of man differs from *G. muris* (Grassi) of the house and albino mouse and *Peromyscus maniculatus*, from *G. microti* (Kofoid and Christiansen), from *Microtus californicus*, and from *G. cuniculi* (Bensen) of the rabbit, in a number of important and readily recognized morphological characters. The *Giardia* of the rat requires further elucidation before conclusions can be drawn regarding it. The following table summarizes the known differences.

Characteristics of Giardia from Man and Rodents

Organ	*enterica*	*muris* Kofoid and Christiansen (1916)	*microti* Kofoid and Christiansen (1916)	*cuniculi* Metzner (1901) Benson (1908)
Shape	Medium slender	Stout	Slender	
Lateral contour of tail	Concave	Convex	More nearly straight	Concave
Level of emergence of anterolateral flagellum	Somewhat anterior	Somewhat posterior	Median	Anterior
Shape of parabasals	Curved comma-shaped	Stout, rounded bodies, often fused	Slender, slightly curved comma-shaped	Stout comma-shaped
Shape of blepharoplasts	Head of axostyle not expanded by them	Head of axostyle expanded	Head of axostyle expanded	?
Anterolateral basal granule	None	Present	Present	?
Posterior basal granule	None	Present	Present	Present

The morphological differences here noted justify the specific distinction of these parasites and raise the question as to the extent, if any, to which the rodent hosts of other species than *G. enterica* can normally serve as carriers and disseminators of the human parasite. We regard Deschien's (1921) conclusion that *Giardia muris* and *G. enterica* are identical species as unsupported by the evidence presented and his morphological analysis as quite inadequate.

It is highly desirable that experiment should determine the extent to which transfer of species among these hosts is feasible and permanent and that the effect of the new hosts upon the structures of the parasites above enumerated should be carefully analyzed before final conclusions as to the status of these species be drawn. The morphological evidence clearly indicates their specific distinction. The status of cross immunities, if any exist, should also be determined.

SUMMARY

Giardia enterica has the typical structures of the genus. It lacks basal granules on the anterolateral and posterior flagella, its blepharoplasts are not enlarged so as to expand the head of the axostyle. The parabasals are rather slender, curved, comma-shaped, tapering, deeply staining structures lying as a pair parallel to each other in various positions posterior to the cytostome in the dorsal region. It has the structurally integrated neuromotor system typical of the genus. The cytostome is an organ of adhesion. There is no evidence of the ingestion of any formed food particles.

There are four chromosomes; a paradesmose forms between parting centrosomes outside of the nuclear membrane which remain intact during mitosis. Daughter parabasals, axostyles, and peristomal fibers are formed by contact outgrowth of the daughter structure or by distad splitting of the parent structures. Anterolaterals are certainly duplicated by new outgrowth.

Mitosis in the cyst resembles that in the free stage except that free flagella are lacking and duplication of the neuromotor elements lags unevenly after nuclear division. Dedifferentiation of free flagella, cytostome, and the entire peristomal fiber occurs in the cyst.

Encystment provides for dispersal of the parasite to new hosts and affords opportunity for multiple mitosis to the 8-zooid, 16-nucleate somatella. Plasmotomy was not seen in the cysts. Binary fission in the cysts was not observed. Interpretations of the cysts as provision for conjugation and for autogamy are erroneous. There is no evidence that there is an *Octomitus* stage (Hartmann, 1910) in the life-cycle of *Giardia*.

Giardia is bilaterally symmetrical. Fission is in the longitudinal, frontal plane. It is asexual reproduction. There is no evidence of a transition through or a return to a one-cell stage. The symmetry of the right side of *Giardia* is that of the one-celled *Chilomastix* and the organs of that side are homologous with those of a *Chilomastix*. The organs of the left side of *Giardia* are those of a *Chilomastix*, but they are those of a morphologically reversed *Chilomastix*, i.e., they form a mirror image of those of the right side. *Giardia* may be derived from *Chilomastix* by a morphological reversal of one daughter nucleus and its attendant neuromotor system at mitosis and the persistence of a

union between the centrosomes via the blepharoplasts forming the anterior commissure. Bilateral symmetry in this instance is brought about by the formation and persistent union of two structural units of opposite symmetries, dextral and sinistral, whose stereometric relations recall those of dextrose and laevulose, and of analogous albuminoids.

Giardia enterica differs in structural details, shape of body, contour of the tail, points of emergence of the anterolateral flagella, shape of the head of the axostyle, absence of basal granules on the flagella, and in shape of the parabasals, from species of *Giardia* in rodents. Rodent species of *Giardia* are therefore not sources of human infection. Experiment is needed to determine the host toleration and cross immunities of *Giardia* in cross infections, the effect of transfer upon the morphological characters, and practicability of rodents serving as temporary or persistent carriers of *Giardia enterica* of man.

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LITERATURE CITED

Benson, W.
1908. Bau und Arten der Gattung *Lamblia*. Zeitschr. f. Hyg. u. Infekt., **61**, 109–114, 6 figs. in text.

Boeck, W. C.
1917. Mitosis in *Giardia microti*. Univ. Calif. Publ. Zool., **18**, 1–26, pl. 1, 1 fig. in text.

Cammidge, P. J.
1914. The faeces of children and adults, their examination and diagnostic significance with indications for treatment (New York. Wood), viii + 576 pp., 15 pls., 96 figs. in text.

Deschiens, R.
1921. Les entérites à *Giardia* (*Lamblia*). Inaug. Diss., Paris, ii + 83 + ii pp., 3 pls.

Doflein, F.
1916. Lehrbuch der Protozoenkunde (ed. 4) (Jena. Fischer), xv + 1190 pp., 1198 figs. in text.

Fantham, H. B., Stephens, J. W. W., and Theobald, F. V.
1916. The animal parasites of man (London. John Bale, Sons & Danielson), xxxii + 900 pp., 324 figs. in text.

Grassi, B.
1881. Di un nuovo parasite dell' uomo *Megastoma entericum* (mihi). Prima nota. Gazetta degli Ospitali, **2**, 575–580.
1882–1883. Sur quelques protistes endoparasites appartenant aux classes des Flagellata, Lobosa, Sporozoa, et Ciliata. Arch. Ital. Biol., **2**, 402–444; **3**, 23–37, pls. 1–4.

Grassi, B., and Schewiakoff, W.
1888. Beitrag zur Kenntnis des *Megastoma entericum*. Zeitschr. f. wiss. Zool., **46**, 143–154, pl. 15.

Hartmann, M.
1910. "Protozoologie" *in* Kisskalt und Hartmann. "Praktikum der Bakteriologie und Protozoologie" (ed. 2). Zweiter Teil, vi + 106 pp., 76 figs. in text.

Hartmann, M., and Schilling, C.
1917. Die pathogenen Protozoen und die durch sie verursachten Krankheiten zugleich eine Einführung in die allgemeine Protozoenkunde. Ein Lehrbuch für Mediziner und Zoologen (Berlin. Springer), x + 462 pp., 337 figs. in text.

Hegner, R. W., and Cort, W. W.
1921. Diagnosis of Protozoa and worms parasitic in man (Baltimore. Johns Hopkins University School of Hygiene and Public Health), 1–72 pp., 8 pls.

Janicki, C.
1911. Zur Kenntnis des Parabasalapparats bei parasitischen Flagellaten. Biol. Centrbl., **31**, 321–330, 8 figs. in text.

Kofoid, C. A.
120. Hookworm and amoebiasis in California. Calif. State Jour. Med., **18**, 329–332.

Kofoid, C. A., and Christiansen, E. B.
1915. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–54, pls. 5–8, 1 fig. in text.

Kofoid, C. A., Kornhauser, S. I., and Plate, J. T.
1919. Intestinal parasites in overseas and home service troops of the U. S. Army with especial reference to carriers of amoebiasis. Jour. Amer. Med. Assoc., **72**, 1721–1724.

Kofoid, C. A., and Swezy, O.
1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad. Arts and Sci., **51**, 289–378, pls. 1–8, 7 figs. in text.
1920. On the morphology and mitosis of *Chilomastix mesnili* (Wenyon), a common flagellate of the human intestine. Univ. Calif. Publ. Zool., **20**, 177–174, pls. 15–17, 2 figs. in text.
1921. The flagellate infections of the human digestion tract *in* Nelson's Loose-leaf Encyclopaedia of Living Medicine, **5**, chap. 20, pp. 365–398, 6 pls. December, 1920.

Metzner, R.
1901. Untersuchungen an *Megastoma entericum* Grassi aus dem Kaninchendarm. Zeitschr. wiss. Zool., **70**, 299–320, pl. 15.

Müller, E.
1890. Ein Fund von *Cercomonas intestinalis* im Jejunum des Menschen. Biol. För. Förh., Stockholm, **2**, 42–54, pl. 2.

Penfold, W. J., Woodcock, H. M., and Drew, A. H.
1916. The excystation of *Entamoeba histolytica* (*tetragena*) as an indication of the vitality of cysts. British Med. Jour., **1916**, I, 714–715 8 figs. in text.

Porter, A.
1916. An enumerative study of the cysts of *Giardia* (*Lamblia*) *intestinalis* in human dysenteric faeces. Lancet, 1916, I, 1166–1169.

Prowazek, S., and Werner, H.
1914. Zur Kenntnis der Flagellaten. Arch f. Schiffs-und Tropenhyg., 18, Beiheft 5, 155–170, pl. 10, 1 fig. in text.

Reuling, F., and Rodenwaldt, E.
1921. *Giardia-Lamblia.* Arch f. Prot., 42, 336–346, 2 figs. in text.

Schaudinn, F.
1903. Untersuchungen über die Fortpflanzung einiger Rhizopoden (Vorläufige Mittheilung). Arb. Kais. Gesundh., 19, 547–576.

Simon, C. E.
1921. *Giardia enterica;* a parasitic intestinal flagellate of man. Amer. Jour. Hyg., 7, 440–491, pls. 22–24, 2 figs. and 12 curves in text. Dated July, published in November.*

Stiles, C. W.
1915. Intestinal infections. The school grades attained by 2166 white school children (1062 boys, 1104 girls) in the city of X, classified by age, sanitation and intestinal parasites. U. S. Public Health Reports, 30, pt. 2, 2060–2067.

Wenyon, C. M.
1915. Observations on the common intestinal Protozoa of man. Lancet, 1915, II, 1173–1183, 1 pl.

Wenyon, C. M., and O'Connor, F. W.
1917. Human intestinal Protozoa in the Near East (London. Wellcome Bur. Sci. Research), 1–218 pp., 4 pls., 5 figs in text.

* Dr. C. E. Simon's article on "*Giardia enterica*, a parasitic intestinal flagellate of man," in the July number of the American Journal of Hygiene (published in November) was received while this article was in press, so that it has not been feasible to incorporate herein a discussion of points raised by his divergent interpretations. A paper dealing with these will appear later.

EXPLANATION OF PLATES

All figures were drawn with camera lucida from specimens of *Giardia enterica* from smears of human stools, fixed in hot Schaudinn's fluid and stained with iron haematoxylin by the wet film method. Magnification of all figures 3200 diameters.

PLATE 23

Fig. 1. Typical active vegetative phase viewed from dorsal surface, showing axostyles, parabasals, cytostomal fibers, intra- and extranuclear rhizoplasts, centrosomes, and full complement of flagella.

Fig. 2. Obliquely lateral view of free flagellate, showing the concave, sucker-like cytostome on ventral face. Note parabasal rhizoplasts extending to the axostyle from the ends of the parabasals.

Fig. 3. Ventrolateral view of active flagellate with cytostome somewhat expanded.

Fig. 4. Dorsal view. Prophase with four chromosomes within the nucleus.

Fig. 5. Ventral view. Prophase with chromatin arranged in an axial spireme; paradesmose present on nucleus at left with the daughter centrosomes at its ends. Note thickened intracytoplasmic flagella.

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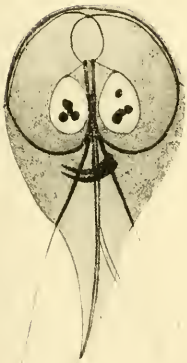

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PLATE 24

All figures of *Giardi enterica* (Grassi) from human stools. × 3200.

Fig. 6. Dorsal view. Nuclei in prophase with four chromosomes in each; centrosomes at opposite poles, each pair connected by a lateral paradesmose. New anterolateral flagella with new anterior node appearing as an anterior outgrowth from the two blepharoplasts and anterior node.

Fig. 7. Ventral view. Further outgrowth of new anterolateral flagella with new cytostomal rim formed posteriorly; equatorial plate in left nucleus while right nucleus is still in prophase; paradesmose visible on each nucleus; parabasals fading out.

Fig. 8. Ventral view. Nuclei in telophase; new intracytoplasmic organelles completed; parabasals indistinct.

Fig. 9. Dorsolateral view from the right showing nuclei in telophase; paradesmose still persisting. Note rhizoplasts running anteriorly from the blunt ends of the parabasals along the dorsal sides of the axostyles. The parabasals are somewhat foreshortened and enlarged.

Fig. 10. Ventral view. Nuclear division completed; new flagella not yet appearing; axostyles, parabasals, posterior peristomal fiber, and nuclei all duplicated.

Fig. 11. Ventral view. Parabasals indistinct; new anterolateral flagella completely formed; posterior peristomal fiber and nuclei duplicated; region of union of axostyles, posterolateral flagella, ventral flagella, and posterior peristomal fiber greatly thickened. Longitudinal fission in frontal plane indicated.

Fig. 12. Dorsal view of two sister individuals in late phase of plasmotomy in frontal plane not yet separated posteriorly. Duplication of organelles completed. Parabasals indistinct.

Fig. 13. Individual in early phase of encystment. The forming cyst has not yet attained its typical ovoidal contour. Cyst wall forming about the partially contracted tail, bilamellate posteriorly.

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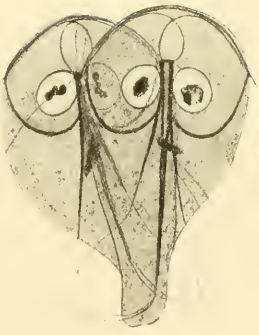

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PLATE 25

All figures of *Giardia enterica* (Grassi) from human stools. × 3200.

Fig. 14. Cyst containing degenerating(?) flagellate. Note persistence of neuromotor system after loss of most of the cytoplasm.

Fig. 15. First division of encysted flagellate; one nucleus still in prophase; parabasals and axostyles duplicated; remnants of intracytoplasmic parts of the flagella persisting.

Fig. 16. Cyst of the spheroidal type. Nucleus on the left showing paradesmose and equatorial plate of the first division. Parabasals greatly enlarged.

Fig. 17. Ellipsoidal cyst. Somewhat abnormal telophase of the first divison in central nucleus; cytoplasmic inclusion at the right (remnant of peristomal fiber?).

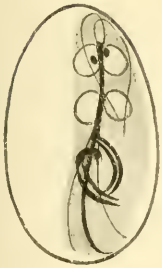
Fig. 18. Ovoidal cyst. Flagellate with first division completed; all nuclei anterior; V-shaped posterior peristomal fibers fading out, their proximal ends shifting posteriorly.

Fig. 19. Asymmetrical irregular cyst. Second division approaching with one nucleus in telophase, one in late prophase, and the others early prophase; four sets of parabasals present.

Fig. 20. Characteristic cysts with four anterior nuclei.

Fig. 21. Greatly elongated cyst with sister flagellates at opposite poles of the common protoplasmic mass and of the cyst.

Fig. 22. Cyst showing the extreme phase of the spheroidal form. Both zooids anterior, parabasals greatly enlarged.



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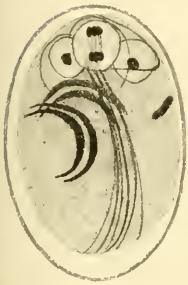

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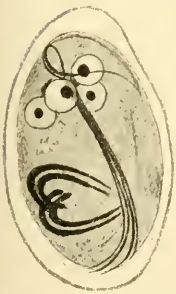

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PLATE 26

All figures of *Giardia enterica* (Grassi) from human stools. × 3200.

Fig. 23. Early prophase of the second division; paradesmose visible on two nuclei; second division of parabasals and axostyles completed; both zooids anterior.

Fig. 24. The daughter zooids diverging to opposite poles in the as yet undivided protoplasm of the cyst. Note coincident divergence of nuclei and neuromotor system in the absence of external free flagella.

Fig. 25. Prophase of the second division with spireme forming in upper two nuclei; one parabasal detached from its mate.

Fig. 26. Prophase of second division with chromosomes doubled in each nucleus, but in pairs, either in the early phase side by side, or in the later one, swinging into an end-to-end relation.

Fig. 27. Second division, two enlarged nuclei in telophase. Note heavy parabasals.

Fig. 28. Second mitosis; division nearly completed. One nucleus still in prophase with doubled chromosomes, the other three nuclei having completed their division. Note variation in size of nuclei, anterior location of all nuclei, and lagging of division in the neuromotor system behind that of the nuclei.

Fig. 29. Second division of nuclei completed. Division of nuclei and neuromotor systems (axostyles and parabasals) synchronous.

Fig. 30. Large asymmetrical cyst with third division in progress showing the nuclei widely divergent in size. Two zooids with larger nuclei, at opposite poles of the cyst.

Fig. 31. Third division partly completed, with twelve nuclei but only a single pair of axostyles.

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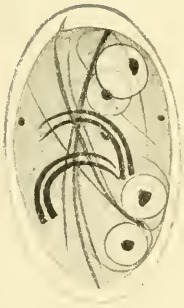

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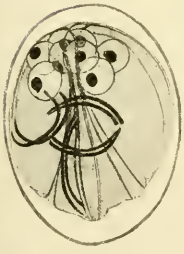

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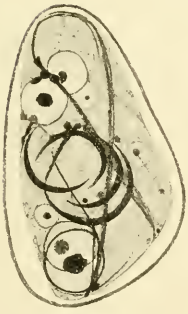

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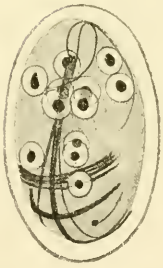

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BY
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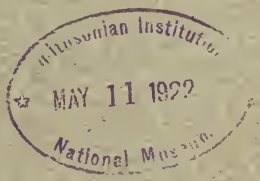

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Vol. 20, No. 9, pp. 235-242 April 12, 1922

THE MICRO-INJECTION OF PARAMAECIUM

BY

CHAS. WM. REES

The development of the technique of isolation and micro-injection by Barber (1914) and of micro-dissection and micro-injection by Chambers (1918) and Taylor (1920) is of fundamental importance. Barber applied this technique to studies in bacteriology; Chambers to studies of the physical changes that occur in living protoplasm, and Taylor demonstrated the presence of conductile fibers in *Euplotes*. The present paper is a report of some studies of the effect of a nematode toxin on *Paramaecium*. The results of feeding *Paramaecium* on *Ascaris* toxin are here contrasted with those of injecting this toxin into the living cytoplasm.

The work was done under the direction of Dr. Charles A. Kofoid of this laboratory and Dr. Walter R. Bloor of the Department of Biochemistry. An attempt was made to induce transmissible changes in the stock of *Paramaecium*. At first, the ciliates were fed on solutions and suspensions of the ground up body wall, of the reproductive organs, and of the digestive tract of *Ascaris* from the pig. The ciliates were also placed in diluted solutions of the body fluid of *Ascaris*. The results in all these tests were negative in so far as toxic symptoms or other noticeable effects are concerned. *Paramaecium* thrived in all the solutions as well as or better than in the controls. An attempt was then made to get more concentrated extracts of the toxins by repeating the work of Shimamura and Fujii (1917).

Shimamura and Fujii extracted from *Ascaris* a proteose toxin which they named askaron. The toxin was obtained as follows. The worms, preserved in alcohol, were dried and pulverized. The fats and lipoids were extracted with ether and alcohol, and the fat free

powder treated with about eight times its volume of water for three two-hour periods in a shaking machine. The water extract was evaporated at 60° C to about one-thirteenth of its volume and two parts of 95 per cent alcohol added to one part of the concentrated extract. A copious precipitate was obtained, giving the phenyl hydrazine reaction and reducing Fehling's solution, but failing to give the iodine test for glycogen. After twenty-four hours the precipitate was filtered out and the filtrate evaporated to a thick syrup at about 50° C. An excess of absolute alcohol added to this syrup produced a precipitate. This precipitate, a white powder, was soluble in water and dilute alcohol. It reacted positively to nearly all of the protein tests. Very small doses of 0.8 mg. injected intervenously into guinea pigs caused respiratory disturbances similar to those of anaphylactic shock and usually produced fatal results within about ten minutes. The post-mortem findings showed inflation of the lungs which is characteristic of anaphylaxis. Horses were even more sensitive than guinea pigs, 0.0004 mg. per kg. of body weight being the lethal dose. Rabbits and dogs were less sensitive, and rats and mice were immune. Similar results were produced by the injection of very large doses interperitoneally into guinea pigs, but in this case the symptoms were not manifest for about six hours. No definite results could be obtained by subcutaneous injection, and the powder was non-toxic when given by mouth.

A substance was prepared by us answering to the description of askaron and giving the same chemical reactions. This substance produced in guinea pigs the chewing movements, gasping for breath, cramps, and partial paralysis described by Shimamura and Fujii, but in none of the five animals tested was the result fatal. The characteristic temperature curve described by these authors was produced in animals surviving the operation. These symptoms were not produced in the three control animals injected with normal salt solution.

Paramaecium thrived in our solutions of askaron. But the following review of the literature on the anatomy and the digestive processes of *Paramaecium* led us to expect this result, since askaron is non-toxic to the experimental mammals when fed to them by mouth. *Paramaecium* has a mouth or cytostome, an elongated cytopharynx, and an anal aperture. According to Nirenstein (1905 and 1920) the food vacuole is produced by an invagination of the lamella which lines the blind caudal end of the cytopharynx, so that the vacuole thus formed is surrounded by a membrane.

The vacuole is filled with bacteria and other food materials which are wafted in by the cytopharyngeal membranelles and is then detached and passed into the endoplasm by a process which resembles a swallowing movement. The food vacuole first becomes tear-shaped by being forced out to a point opposite the attached end. It is then rotated through an angle of 180°, being in the meantime drawn out at the attached end, so that when detached it is spindle shaped, but quickly becomes spherical.

There is a forward streaming of the endoplasm and its inclusions along the animal's left side from the posterior to the anterior end and caudad along the right side. The food vacuole begins this circuit but goes only halfway, crossing over at a point opposite the posterior end of the macronucleus and returning in the posteriorly streaming protoplasm of the right side to the starting point. It usually repeats this circuit several times.

It was demonstrated by the use of very dilute solutions of neutral red that the reaction of the vacuole when first released from the cytopharynx is acid. During this time endoplasmic granules, which at first adhere to the vacuolar membrane, pass through the latter into the vacuole. They are also stained red. These granules and the ingested organisms are then aggregated into a compact mulberry mass, surrounded by a clear fluid within the vacuole. The fluid is gradually resorbed, so that the vacuole becomes smaller until its membrane comes into immediate contact with the mulberry mass. Then the vacuole begins to get larger, the mulberry mass is disrupted, and the endoplasmic granules and the bacteria become again sharply differentiated. This marks the close of the first period.

The microörganisms within the vacuole and the vacuolar fluid now become colorless. The granules retain their color until finally dissolved. These changes characterize a transition from an acid to an alkaline reaction. No further changes were recorded until the vacuoles become aggregated in the region of the anal aperture and are defaecated.

Nirenstein concluded, in agreement with Greenwood (1894), and Greenwood and Saunders (1894), and in opposition to Metchnikoff (1889), that no digestion takes place during the time of the acid reaction. But Metalnikow (1912) found that the acid reaction continues longer and is more pronounced when protein is fed to *Paramaecium* than when carbohydrates are fed. Lund (1914) proved that in *Bursaria* protein digestion occurs during the acid reaction.

In 1910 Nirenstein demonstrated by the use of Sudan III that fats are digested, in *Paramaecium*, during the time of the alkaline reaction. Fat-containing vacuoles appeared in organisms that were first starved, then placed in dilute solutions of glycerol and sodium oleate. Meissner (1888) demonstrated that starch is digested and stored as glycogen in the Protozoa.

The above account shows that food of *Paramaecium* is separated from the endoplasm by a membrane just as truly as is the food in the intestine from the adjoining protoplasm in the Metazoa, where the digestive tract has a continuous lumen. Furthermore, the digestive processes of the Protozoa are in fundamental respects similar to those of the Metazoa. It was thought, therefore, that askaron might be found toxic to *Paramaecium* when injected into the living cytoplasm.

In order to accomplish micro-injection, a special method of isolation was devised (Rees, 1921). Barber's micro-injection pipette (Barber, 1914) was used.

One of the surprising observations of this work was the exceeding toughness of the pellicle of *Paramaecium*. Organisms were frequently bent double on the point of the pipette and the pellicle still not pierced. It was always easy, however, to determine whether or not the injection was successful. A characteristic flash (gelation?) is seen in the cytoplasm, caused by the disturbance of the protoplasmic granules and other inclusions. If this disturbance extends to the anterior end of the body, the animal disintegrates. It may extend over the entire posterior half of the body, as it usually does in successful injections, and then the organism recovers.

At first the askaron used in injection was dissolved in normal salt solution as it is when injected into mammals. But the controls showed that injection of the normal salt solution alone was fatal to *Paramaecium*. It was then dissolved in tap water, and this solution *Paramaecium* survived.

The results of the injections and controls are recorded in the following tables. Table I shows that, of fifteen organisms successfully injected with normal salt, seven were dead in fifteen minutes and the other eight in thirty minutes. As is shown by Table II ten organisms successfully injected with tap water were all alive and normal at the end of three hours but they dried out overnight. Eight others injected with tap water, as shown in Table III, were normal at the end of one hour. The injections with askaron are recorded in

Table IV. Of the nine successfuly injected, one was dead in thirty minutes, eight were normal at the end of one hour, and four at the end of twelve hours.

The injected fluid comes in contact not only with the endoplasm but also with the ectoplasm. The dose was not constant but was equal in volume to about one-fifth of the body of *Paramaecium*. The path followed by this injected fluid was, therefore, in part different from that taken by fluid entering the food vacuoles through the cytopharynx. Unlike injected droplets of mercury which were soon east out of the body, the injected fluids were retained at least during the five to fifteen minutes of careful observation immediately after the injection.

TABLE I

Injection with Normal Salt Solution

No. of the Organism	Result of Injection	Effect on Organism		
		15 minutes	30 minutes	12 hours
1	Unsuccessful	Normal	Normal	
2	Successful	Dead		
3	Successful	Dead		
4	Successful	Very feeble	Dead	
5	Successful	Dead		
6	Control	Normal	Normal	
7	Successful	Dead		
8	Successful	Dead		
9	Successful	Normal	Dead	
10	Successful	Normal	Dead	
11	Successful	Normal	Dead	
12	Successful	Very feeble	Dead	
13	Successful	Very feeble	Dead	
14	Successful	Very feeble	Dead	
15	Successful	Lost		
16	Successful	Dead		
17	Successful	Dead		
18	Control	Normal	Normal	
19	Successful	Dead		
20	Successful	Dead		

TABLE II

Injection with Tap Water

No. of the Organism	Result of Injection	Effect on the Organism				
		15 minutes	30 minutes	1 hour	3 hours	12 hours
1	Successful	Normal	Normal	Normal	Normal	Dried out
2	Successful	Normal	Normal	Normal	Normal	Dried out
3	Successful	Normal	Normal	Normal	Normal	Dried out
4	Successful	Normal	Normal	Normal	Normal	Dried out
5	Successful	Normal	Normal	Normal	Normal	Dried out
6	Disrupted by too heavy dose	Dead				
7	Successful	Normal	Normal	Normal	Normal	Dried out
8	Successful	Normal	Normal	Normal	Normal	Dried out
9	Successful	Normal	Normal	Normal	Normal	Dried out
10	Successful	Normal	Normal	Normal	Normal	Dried out
11	Successful	Normal	Normal	Normal	Normal	Dried out
12	Control	Normal	Normal	Normal	Normal	Dried out

TABLE III

Injection with Tap Water

No. of the Orgauism	Result of Injection	Condition of the Organism			
		15 minutes	30 minutes	1 hour	12 hours
1	Successful	Normal	Normal	Dried	No record
2	Successful	Normal	Normal	Normal	No record
3	Successful	Normal	Normal	Normal	No record
4	Disrupted by too heavy dose	Dead			
5	Disrupted by too heavy dose	Dead			
6	Successful	Normal	Normal	Normal	No record
7	Successful	Normal	Normal	Normal	No record
12	Successful	Normal	Normal	Normal	No record
18	Successful	Normal	Normal	Normal	No record

TABLE IV

Injections with Askaron 4 mg. per cc. in Tap H_2O

No. of the Organism	Result of Injection	Condition of the Organism			
		12 minutes	30 minutes	1 hour	12 hours
1	Disrupted by too heavy dose	Dead			
2	Disrupted by too heavy dose	Dead			
3	Successful	Normal	Normal	Normal	Normal
4	Successful	Normal	Normal	Normal	Normal
5	Successful	Normal	Normal	Normal	Normal
6	Successful	Normal	Normal	Normal	Dead
7	Successful	Normal	Normal	Normal	Dead
8	Disrupted by too heavy dose	Dead			
9	Successful	Normal	No record		
11	Successful	Lost			
12	Successful	Lost			
13	Control	Normal	Normal	Normal	Dead
14	Control	Normal	Normal	Normal	Dead
15	Control	Normal	Normal	Normal	Dead
16	Control	Normal	Normal	Normal	Normal

CONCLUSIONS

1. Micro-injection has been used in a comparative study of the effects of the toxins of *Ascaris* on *Paramaecium* when taken in through the cytostome and when introduced directly into the living cytoplasm by the pipette.

2. It has been found that the solution of askaron is not fatal to *Paramaecium* when ingested through the cytostome, and it is no more fatal when injected into the living cytoplasm than is its solvent.

LITERATURE CITED

Barber, M. A.

1911. Technique for inoculation into living cells. Jour. Inf. Diseases, **8**, 348–360, 5 figs. in text.

1914. The pipette method in the isolation of single microörganisms and in the inoculation of substances into living cells. Philippine Jour Sci., **9**, Ser. B, 307–385, 19 figs. in text.

Chambers, R.

1918. The micro-vivisection method. Biol. Bull., **34**, 120–136, 8 figs. in text.

Greenwood, M.

1894. On the constitution and mode of formation of food vacuoles in Infusoria as illustrated by the history of the process of digestion in *Carchesium polypodium*. Phil. Trans. Roy. Soc. London, **185**, B, 355–383, pl. 1.

Greenwood, M., and Saunders, E. R.

1894. On the rôle of acid in protozoan digestion. Jour. Physiol., **16**, 441–467, pl. 15.

Lund, E. J.

1914. The relation of *Bursaria* to food. Jour. Exp. Zool., **17**, 1–43, pls. 1–2.

Meissner, M.

1888. Beiträge zur Ernährungsphysiologie der Protozoen. Zeitschr. f. wiss. Zool., **46**, 498–516, pl. 1.

Metalnikow, S.

1912. Contributions à l'étude de la digestion intracellulaire chez les Protozoaires. Arch. de Zool. Exp. et Gen. (5), **9**, 373–499, pls. 21–22.

Metchnikoff, E.

1889. Recherches anatomiques et physiologiques sur les infusoires cilies. Ann. Inst. Pasteur, **3**, 25–29.

Nirenstein, E.

1905. Beiträge zur Ernahrungsphysiologie der Protisten. Zeitschr. allg. Physiol., **5**, 435–510, pl. 1.

1910. Ueber Fettverdauung und Fettspeicherung bei Infusorien. Zeitschr. f. allg. Physiol., **10**, 137–149, pl. 1.

1920. Ueber das Wesen der Vitalfärbung. Pflügers Arch. f. ges. Physiol., **179**, 233–337, pl. 1.

Rees, C. W.

1921. The technique of isolation of *Paramaecium* with notes on micro-injection and micro-dissection. (In press.)

Shimamura, T. and Fujii, H.

1917. Ueber das Askaron, einen toxischen Bestandteil der *Helminthen* besonders der *Askariden* und seine biologische Wirkung. Jour. Coll. Agr. Imp. Univ. Tokyo, **3**, 189–257.

Taylor, C. V.

1920. Demonstration of the function of the neuromotor apparatus in *Euplotes* by the method of micro-dissection. Univ. Calif. Publ. Zool., **19**, 403–470, pls. 29–33, 2 figs. in text.

1920. An accurately controllable micro-pipette. Science (n. s.), **51**, 617–618, 1 fig. in text.

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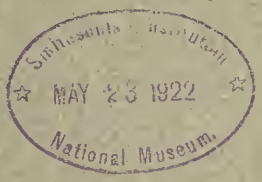
BY
J. DALEY McDONALD



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CONTENTS

	PAGE
Introduction	244
Acknowledgments	245
Material and technique	245
Occurrence and geographic distribution	246
Studies of living organisms	246
Systematic position of genus and species	248
Balantidium coli	249
Balantidium suis sp. nov.	250
Method and use of measurements	254
Other specific characters	258
Balantidium from man	261
Morphology	262
Ectoplasmic structures	263
Pellicle	263
Ectoplasm	266
Cilia	270
Basal apparatus of cilia	271
Ciliary movements	273
Cytostome	276
Oral plug	280
Contractile vacuoles	280
Endoplasmic structures	282
Endoplasm	282
Food vacuoles	282
Macronucleus	282
Micronucleus	283
Neuromotor apparatus	284
Motorium	285
Circumoesophageal fiber	285
Adoral ciliary fiber	286

PAGE
Adoral ciliary rootlets 286
Radial fibers 286
Discussion 288
Summary 293
Literature cited 294
Explanation of plates 298

INTRODUCTION

The earliest observation of Protozoa of the genus *Balantidium* has in several instances been accredited to Antony von Leeuwenhoek (1708). During an attack of dysentery he detected motile organisms in the discharges. At that time no discrimination had been made between ciliated and flagellated protozoa and his account of his observations is not sufficiently complete to make possible the classification of the organisms which he found. However, he stated that they were about the size of red blood corpuscles, which would indicate that they were intestinal flagellates and not *Balantidium*, which is very much larger.

Malmsten (1857) was the first to describe *Balantidium coli*. This species has become better known than the other species of the genus, due to its being the cause of a specific dysentery known as balantidiasis. Two persons suffering from this disease came to Malmsten for medical attention during 1856–57. He was assisted in the study of protozoans which he found in the excreta from these two patients by the zoologist Loven who believed that the parasites were new to science and so prepared a careful description of them accompanied by figures. For the organism they suggested the name *Paramoecium* (*?*) *coli*. Since that time infections with *Balantidium coli* have been reported in increasing numbers and some cytological studies have been made, though much more attention has been given to the problems of prophylaxis and treatment of the disease which this species causes than to the parasite itself.

The first record of *Balantidium coli* as a parasite of pigs was made by Leuckart (1861). Stein (1862) also studied these forms from pigs and he was the first to assign them to the genus *Balantidium*. The genus had been established by Claparède and Lachmann (1858) with *Balantidium entozoön* from the frog as the type species. More recently Strong (1904), Brumpt (1909), Walker (1913), and others have carried on investigations on this parasite of pigs in order to become acquainted with the problems involved in the infection of man.

ACKNOWLEDGMENTS

It has been my privilege to study the morphology of *Balantidium coli* and *Balantidium suis* (sp. nov.) under the direction of Professor Charles A. Kofoid, to whom I am indebted for helpful suggestions and for oversight of the entire work. Acknowledgment is due Professor William W. Cort for many valuable criticisms. I also take this opportunity to express my appreciation of the courtesy of Mr. R. B. Brown, superintendent of the Oakland Meat and Packing Company, who kindly granted me permission to work in the company's *abattoir* and also facilitated the work in every possible way.

MATERIAL AND TECHNIQUE

The material for these studies was obtained almost exclusively from pigs killed by the Oakland Meat and Packing Company, Stockyards, California. At their *abattoir* I was permitted to work in the room where the pigs were dressed, which made it possible to obtain the material from the intestine before it had cooled below the normal body temperature. To determine the presence of the balantidia a small slit was made in the caecum and a drop of the contents withdrawn with a pipette. This drop was quickly placed on a warm slide and examined with a microscope. If the animals were present they would be detected very readily for they are exceedingly active; in most cases they occurred in numbers sufficiently large that from one to ten could be seen in every field when a 16 mm. objective was used. This method was rapid enough to allow all pigs to be examined as fast as they were killed and dressed.

A sample from the caecum was not relied upon as critical in the determination of infection until examination of the entire length of the intestine had been made in several instances. In order to discover the normal distribution throughout the intestine it was removed entire and taken to the laboratory of the *abattoir*. Incisions were made every one or two feet, beginning with the duodenum and continuing to the rectum, and samples examined from each of these incisions. In no cases were balantidia found more than three feet above the ileocaecal valve, and only in two or three instances were any at all present in

the small intestine. In the caecum and first three or four feet of the colon the balantidia were always more active and more numerous than elsewhere. Posteriorly from this region they were found in progressive stages of encystment until in the rectum the majority were completely encysted.

Occurrence and Geographic Distribution

Approximately 200 pigs were examined. They had been raised in the Sacramento Valley, except for one lot from Los Banos, California, and a lot from the state of Nevada. Of the 200 pigs examined 68 per cent were infected. The examinations were made at nine separate times between September, 1913, and May, 1918, ten to sixty individuals being examined each time. In five of the nine lots every pig was found to be infected. The lowest percentage of infection was 13 per cent, in the lot shipped from Nevada. This indicates a very general infection of pigs with *Balantidium* in this region of the United States.

Stiles (cit. Strong, 1904), Bel and Couret (1910), and others have previously found the organisms in pigs in the United States. Leuckart (1861), working in Germany, was the first to find *Balantidium* in pigs. Since then Stein (1862), Eckecrantz (1869), and Prowazek (1913) have reported them from the same country. In 1871 Wising noted their occurrence in pigs in Sweden. Grassi (1882) and Calandruccio (1888) have found the parasites in swine in Italy. Rapchevski (1882) reported the occurrence of balantidia in Russia. In France they have been found in pigs by Railliet (1886), Neumann (1888), and Brumpt (1909). Strong (1904), Walker (1913), and several others have noted the occurrence in pigs in the Philippine Islands. Similar reports from China have been made by Maxwell (1912), and Mason (1919); from Cuba by Taboadela (1911); and from South America by Bayana (1918). These citations indicate that *Balantidium coli* is probably as widely distributed geographically as is its host, the pig.

Studies of Living Organisms

The balantidia are very sensitive to changes of temperature. When the medium in which they are swimming is cooled a few degrees they slow up their movements very decidedly. After a time they become almost perfectly spherical, in which form their activity is restricted to a rotary motion with little or no progression. In this condition they will live for six or eight hours at ordinary room temperature.

In order to avoid the deleterious effects caused by cooling and by increased bacterial action, most of the studies on living organisms were made at the *abattoir*. When continuous observation over a long period was desired, however, the material was conveyed to the laboratory in thermos bottles and kept in the incubator at 37.5° C. In this manner material could be kept for three days. Ultimate degeneration of the organisms seemed to be due more to the increase in the bacterial content of the medium than to any other cause. During observation either an electric warm stage or the microscope warm oven designed by Long (1912) was employed. Of the several vital stains used, neutral red proved most satisfactory in the differentiation of the neuromotor apparatus.

Fixation and staining.—The following fixatives were used: Schaudinn's fluid, Zenker's fluid, formalin, osmic acid, and pieromercuric fluid (according to the formula by A. D. Drew, used by Yocom, 1912). Quick action was one of the most important factors in the fixation, and was usually obtained by having the killing fluid hot (60–80° C.) and using an amount at least equal to the amount of the material to be fixed. Frequently the action was so nearly instantaneous that the cilia on the killed animals retained their exact relative position (see fig. N). After fixation the material was thoroughly washed, iodine alcohol being used if mercurial salts were present. Material was preserved in 70 per cent alcohol.

Before staining, the preserved material was usually concentrated by elimination of lighter débris by centrifuging and the heavier by sedimentation. Water was found to be a more satisfactory medium for these operations than either alcohol or salt solutions. In case sections were to be made, additional care was taken in the concentration process and then the material was handled according to the methods employed by Metcalf (1909) and by Sharp (1914).

Iron haematoxylin gave uniformly the best results in staining. For cysts, however, on account of their imperviousness, it was necessary to use Delafield's haematoxylin to which had been added a small amount of acetic acid. In addition to the first mentioned stain, Mallory's connective tissue stain was used on sections.

SYSTEMATIC POSITION OF GENUS AND SPECIES

Claparède and Lachmann (1858) removed *Bursaria entozoön* from the genus in which it had been placed by Ehrenberg (1838) and created for it the new genus *Balantidium*. This genus was of the family Bursaridae and the order Heterotricha. The twenty-two species of the genus that have been described to date are listed below.

KNOWN SPECIES OF THE GENUS BALANTIDIUM

Species	Original description by	Hosts
Balantidium entozoön	Ehrenberg, 1838	Rana esculenta
		Rana temporaria
Balantidium coli	Malmsten, 1857	Sus serofa
		Homo sapiens
Balantidium duodeni	Stein, 1862	Rana esculenta
Balantidium elongatum	Stein, 1862	Triton cristatus
		Triton alpestris
		Triton marmoratus
		Rana esculenta
		Rana temporaria
Balantidium medusarum	Mereschkowsky, 1879	Bougainvillea, Obelia, Eucope, Broda sp.?
Balantidium amphictenides	Entz, Sr., 1888	Amphictenis, Turbellaria marina
Balantidium gyrans	Kellicott, 1889	Aquatic worm
Balantidium viride	Willach, 1893	Columba sp.?
Balantidium minutum	Schaudinn, 1899	Homo sapiens
Balantidium giganteum	Bezzenberger, 1903	Rana esculenta
Balantidium helenae	Bezzenberger, 1903	Rana cyanophlyctis
		Rana tigrina
		Rana limnocharis
		Rana hexadactyla
Balantidium gracile	Bezzenberger, 1903	Rana cyanophlyctis
		Rana hexadactyla
Balantidium rotundum	Bezzenberger, 1903	Rana esculenta
Balantidium falciformis	Walker, 1909	Rana palustris
Balantidium ovale	Dobell, 1910	Rana tigrina
Balantidium hyalinum	Dobell, 1910	Rana tigrina
Balantidium littorinae	Chagas, 1911	Littorina
Balantidium testudinis	Chagas, 1911	Testudo graeca
Balantidium hydrae	Entz, Jr., 1913	Hydra olygactis
Balantidium piscicola	Entz, Jr., 1913	Piarectus brachypomus
Balantidium caviae	Neiva *et al.*, 1914	Cavia aperea
Balantidium orchestia	Watson, 1916	Orchestia agilis
		Talorchestia longicornis

The wide diversity of hosts, ranging from hydroids and crustaceans to the warm-blooded vertebrates, including man, must demand a wide versatility on the part of the parasite. Considerable structural variation is apparent even on cursory examination, and some of these structural differences might be sufficiently marked to serve for generic differentiation. A new generic division would seem desirable, but the suggestions of Bütschli (1884) and Schweier (1900) in this direction have not been generally accepted.

Balantidium coli Malmsten (1857)

SYNONOMY:

Paramoecium (?) coli Malmsten, 1857.
Plagiotoma coli, Claparède and Lachmann, 1858.
Leucophyra coli, Stein, 1860.
Holophyra coli, Leuckart, 1861.
Balantidium coli, Stein, 1862.

Up to the present time only one species, *Balantidium coli,* has been described as parasitic in pigs. It was first described by Malmsten (1857) who, noting its likeness to *Paramoecium colpoda* (Ehrenberg), suggested the name *Paramoecium (?) coli.* During the following year, Claparède and Lachmann (1858) reproduced one of Malmsten's original figures and after considering his description transferred the species to the genus *Plagiotoma.* In 1860, Stein, using the description by Malmsten (1857), pointed out that the organism was not a *Paramoecium* and believed that it properly belonged in the genus *Leucophyra.* Leuckart in 1861 discovered a ciliate in the intestine of pigs which he concluded was identical with the one already described, but he was not satisfied with the genus to which it had been assigned by Stein (1861) and believed that its closest relation was with *Holophyra* in which genus it should be placed. In 1863 he still retained this view but suggested the appropriateness of the establishment of a new genus. But Stein (1862) had already recognized those characters of the species which showed its close relation to *Balantidium entozoön* and had placed it in the genus *Balantidium.*

During the present investigation the following specific characteristics have been found very constant. The individuals of the species *Balantidium coli* are ovoid in form, the more pointed end being anterior; length varies from 30μ to 150μ; breadth varies from 25μ to 120μ; in the majority of individuals the length is 1.3 times the breadth; the greatest transverse diameter intersects the longitudinal axis poster-

ior to its midpoint; the adoral zone is approximately terminal, and the anterior tip of the body lies within it; the plane of demarcation between the apical cone of the ectoplasm and the endoplasm is approximately at right angles to the long axis of the body; the macronucleus is elongate but the length usually does not exceed three times the breadth; two contractile vacuoles are present, a smaller one located anteriorly and a larger one located posteriorly; a posterior cytopyge is usually distinctly visible.

Balantidium suis sp. nov.

Early in the work of examining pigs for *Balantidium coli* it became evident that this protozoan showed extreme variation in shape. In many instances the diversity occurred among individuals from the same host. Further observations led me to believe that there were two fairly distinct types, the one, longer and more slender as compared with the other which was distinctly ovoid. Measurements have been recorded by various writers of *Balantidium coli* from man. Malmsten (1857) in the original description gave the length as 60–100μ; breadth, 50–70μ. Solojew (1901) recorded the length as 65μ, the breadth as 40μ. Wising (1871) states that the length varies from 50–100μ, while the breadth varies from 40–50μ. Prowazek (1913) gave the length as 52–71μ, the breadth, 40–58μ. Leuckart (1861) measured balantidia from swine and found the length to be 75–110μ and the breadth 70μ. Still others give dimensions, but all are inadequate for the determination of the occurrence of types with distinct proportions. First, with one or two exceptions all dimensions have been taken of balantidia found in man, and from these it might not be safe to draw conclusions regarding diversity among those found in pigs. In the second place, the measurements given are either averages or else represent extreme limits. In either case they are practically useless in determining individual variations, for even in the case of extreme types the range between limits is so great that two, or even more, distinct types, based on proportions of breadth to length, might be included. Nowhere has there been found a series of individual measurements which would make it possible to determine whether variations were continuous or discontinuous. To obtain such a series of measurements was the purpose of the phase of the work about to be described.

Material which was to be used in taking the measurements was killed and preserved with all possible care. Hot Schaudinn's fluid was used in all cases, the material being quickly and thoroughly mixed

into a large quantity of it so that action would be as nearly instantaneous as possible, thus avoiding distortion. Osmic acid vapor was tried but Schaudinn's fluid gave equally good results and was more convenient for manipulation. Material was never allowed to cool before fixing, for on cooling the individuals tend to become spherical. Several attempts were made to measure living organisms but their ceaseless activity at normal temperature (37.5° C) made this almost impossible and slowing them up by the use of Irish moss or by cooling, as mentioned above, caused them to become distorted. If there were changes due to fixation, the logical expectation would be that the error would be on the side of conservatism for such changes would tend to obliterate rather than accentuate the division into two groups; for the shape of the elongate forms would be more changed by the fixative, the tendency being for them to shorten and broaden and thus approach the ovoid type. However, in the method of fixation used, I am sure that distortion was so slight as to be negligible.

Following fixation the material was carefully washed and carried slowly through the lower grades of alcohol to 70 per cent in which the material was kept for measuring. A drop of the material from which measurements were to be taken was placed on a slide, covered with a coverglass, the excess of fluid removed, and the edges sealed with vaseline to prevent evaporation. Just enough fluid was removed from under the coverglass to reduce the depth of the medium so that the majority of the animals would lie flat, and yet not enough to allow the coverglass to exert any pressure. The exertion of pressure on the animals, however, would ordinarily be prevented by the presence of large particles of foreign material. The object of having animals lie flat on the slide was to avoid the error which would otherwise be caused by foreshortening. A slight elevation of one end would make considerable error in the determination of the length of the animal.

The slide was then placed on the microscope and systematically examined by the use of the mechanical stage. Beginning at the upper left-hand corner and progressing as one would in reading a book, every individual encountered in the survey was measured. The only exceptions made were in case the animal was not lying flat or showed marked signs of distortion. This procedure avoided selection which might unconsciously be made by the observer. For making the measurements a 4 mm. objective was used in combination with an ocular-micrometer inserted in 9x compensating ocular. With the magnification given by this combination the limit of error did not exceed one micron.

The longitudinal axis and the longest transverse axis of each individual were measured, and the ratio of length to breadth computed (see Table I).

TABLE I

Comparative Measurements of One Hundred Individuals Each of Balantidium coli and Balantidium suis

Individuals from Pig No. 1 (*Bal. suis*, with five exceptions)			Individuals from Pig No. 4 (*Bal. coli*, with one exception)		
Length in microns	Breadth in microns	Ratio of length to breadth	Length in microns	Breadth in microns	Ratio of length to breadth
114	42	2.71	99	75	1.32
108	51	2.11	96	81	1.19
63	36	1.75	126	75	1.68*
90	45	2.00	118	87	1.36
93	48	1.94	111	87	1.28
72	39	1.85	87	75	1.16
126	69	1.83	90	66	1.36
87	48	1.82	105	75	1.40
108	57	1.90	78	66	1.18
102	54	1.89	90	63	1.43
117	51	2.30	87	72	1.21
120	57	2.10	87	72	1.21
96	42	2.29	99	75	1.32
84	39	2.12	105	75	1.40
120	48	2.50	89	75	1.19
117	48	2.42	81	63	1.28
93	51	1.83	81	66	1.23
96	51	1.89	75	63	1.19
72	57	1.26*	84	69	1.22
75	54	1.39*	69	60	1.15
111	51	2.14	90	66	1.36
111	54	2.03	93	75	1.23
84	36	2.31	87	69	1.26
69	39	1.77	90	81	1.11
78	51	1.52*	105	90	1.16
81	45	1.80	87	69	1.26
111	45	2.42	84	72	1.17
81	45	1.80	66	58	1.14
84	45	1.86	75	60	1.25
90	45	2.00	81	63	1.29
84	42	2.00	81	60	1.35
90	42	2.12	99	78	1.27
117	57	2.03	87	69	1.26
60	33	1.82	78	63	1.24
108	57	1.90	81	60	1.35
108	57	1.90	105	75	1.40
96	48	2.00	90	60	1.50
75	42	1.79	93	78	1.18

* Other characters showed that these individuals were of the other species represented in the table.

TABLE 1—(*Continued*)

Individuals from Pig No. 1 (*Bal. suis*, with five exceptions)			Individuals from Pig No. 4 (*Bal. coli*, with one exception)		
Length in microns	Breadth in microns	Ratio of length to breadth	Length in microns	Breadth in microns	Ratio of length to breadth
105	57	1.85	69	60	1.15
99	57	1.74	81	54	1.50
105	54	1.95	87	66	1.32
54	27	2.00	84	69	1.22
57	30	1.90	78	60	1.30
36	24	1.50*	66	51	1.29
81	31	2.23	66	54	1.22
75	42	1.78	96	66	1.45
66	33	2.00	81	54	1.50
78	36	2.08	84	66	1.28
93	39	2.39	114	87	1.31
63	36	1.74	87	69	1.26
102	48	2.06	93	72	1.29
78	39	2.00	84	72	1.17
81	42	1.93	102	65	1.58
87	48	1.82	87	63	1.38
93	45	2.03	99	66	1.50
84	42	2.00	81	72	1.13
81	39	2.04	78	63	1.24
75	39	1.93	102	75	1.36
78	45	1.74	66	48	1.38
75	37	2.01	75	57	1.32
100	45	2.11	84	66	1.27
81	39	2.04	93	66	1.40
72	39	1.85	72	58	1.24
84	40	2.06	96	72	1.33
90	42	2.07	90	68	1.32
78	36	2.08	60	39	1.54
102	48	2.06	87	69	1.26
81	37	2.10	69	54	1.28
66	37	1.78	90	69	1.31
87	36	2.41	69	54	1.28
78	42	1.86	72	60	1.20
84	42	2.00	75	57	1.47
90	38	2.37	75	62	1.21
66	35	1.89	72	63	1.14
78	42	1.86	90	75	1.20
97	44	2.20	74	66	1.12
79	37	2.07	72	54	1.33
98	42	2.32	60	50	1.20
90	45	2.00	84	58	1.45
87	49	1.78	84	64	1.31
90	36	2.50	88	60	1.47
81	35	2.31	99	75	1.32
69	48	1.44*	75	54	1.39

* Other characters showed that these individuals were of the other species represented in the table.

TABLE I—(*Continued*)

Individuals from Pig No. 1 (*Bal. suis*, with five exceptions)			Individuals from Pig No. 4 (*Bal. coli*, with one exception)		
Length in microns	Breadth in microns	Ratio of length to breadth	Length in microns	Breadth in microns	Ratio of length to breadth
90	54	1.68	81	69	1.18
66	33	2.00	66	52	1.27
76	38	2.00	93	63	1.48
78	42	1.86	81	75	1.08
90	57	1.58	96	75	1.28
66	40	1.65	114	90	1.27
81	39	2.04	75	60	1.25
96	39	2.43	87	72	1.21
75	38	1.98	84	60	1.40
84	39	2.18	87	63	1.38
51	30	1.70	84	63	1.33
75	39	1.92	72	60	1.20
84	40	2.05	90	66	1.36
116	47	2.48	84	72	1.16
84	36	2.32	93	72	1.29
81	39	2.04	87	58	1.50
75	45	1.68	93	72	1.29
Average 86	43	1.99	86	66	1.30

While taking the measurements of each individual, observations were made regarding the position of the mouth, the type and size of macronucleus, number and location of contractile vacuoles, and any other characters which might aid in differentiation.

In the handling of the data on dimensions I have followed in a general way the method used by Jennings (1908) in differentiating races of *Paramaecium*. For the purpose of this work, however, the results seemed more lucid if, instead of plotting length and breadth along separate axes, the ratio of length to breadth was computed for each individual and if these ratios were then plotted on the abscissa while the numbers of individuals having each of these ratios were plotted on the ordinate. In computing the ratios the quotient was carried to the second decimal place. But in the construction of the curves only intervals of tenths (or first decimal place) were used; thus, for example, all ratios occurring between 1.25 and 1.34 inclusive were grouped as if they were 1.3. This had two advantages: first, it produced a smoother, steeper curve than would result if smaller intervals were taken, using the same number of individuals measured, and emphasized group rather than individual variations. Second, this grouping reduced any error which might result from the observer showing a preference for one graduation of the micrometer when

an individual measured more than one but less than another whole division of the scale; e.g., such a preference might result in the tabulation of several individuals having a length of 72μ, and of none with a length of 71μ, though in reality all lay within these two limits and as many were as near to one as to the other.

As previously mentioned, the graduations along the ordinate represent the number of individuals, each small interval representing one individual. In Jennings' (1908) work these intervals represent percentages of the total number of individuals. But it happens that

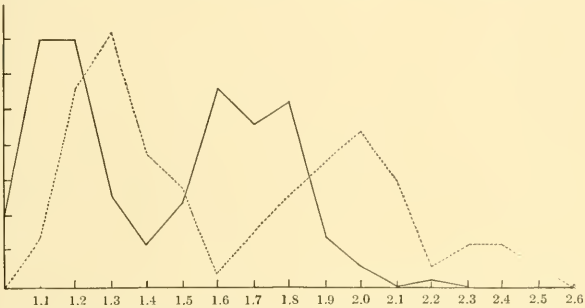


Fig. A. Graphic representation of the variation in the ratio of length to breadth among 200 *Balantidium* chosen at random from samples of material taken from several different pigs. The number of individuals is measured on the ordinate, the ratios on the abscissa. The dotted line is the curve resulting from the combination of the two curves shown in figure B, superimposed here to facilitate comparison.

in the graphs shown in figures B and C, the number of individuals showing a certain ratio is identical with the percentage of the total, for in these cases the total is 100 individuals.

Figure A represents graphically the result of the first attempt to determine the existence of different types. Measurements were made of 200 individuals. At least ten slides were used in getting these measurements and they were prepared from samples taken from nearly as many different pigs. It will be noted that the curve produced by plotting the ratios of these individuals is decidedly bimodal. One mode represents those individuals which are approximately 1.2 times as long as wide, while the other represents those which are 1.6 to 1.8 times as long as wide.

These findings seemed to fully justify my early suspicions that there were two very different types of balantidia parasitic in pigs.

But it was decided to conduct one more experiment, for corroboration, under slightly different conditions and with especial care in fixation of the material. It had been noted that though often both types occurred in the same pig (which was the case in most of the samples used in the first measurements), still one type might be greatly in excess, or there might be only one type present. In getting material

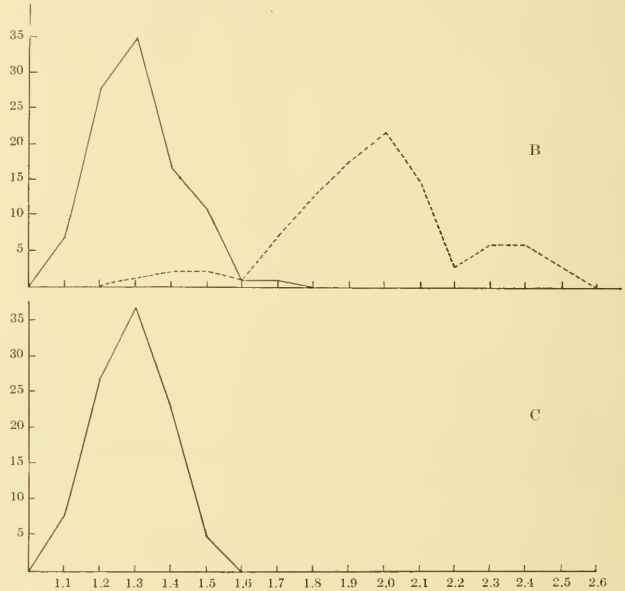


Fig. B. Graphic representation of the difference in ratio of length to breadth between two carefully selected lots, of 100 individuals each, of *Balantidium* from separate hosts. The continuous line represents those from one pig (nearly all are *Balantidium coli*); the broken line, those from the other pig (nearly pure infection with *Balantidium suis*).

Fig. C. This graph shows the variation in the ratio of length to breadth among 100 *Balantidium* secured from a case of balantidiasis in man.

for this second set of measurements, it seemed best to take it from pigs which had, as nearly as could be determined, pure infections of the respective types. Fortunately these requirements were fulfilled in the next lot of pigs examined. Both samples of material, the one containing the ovoid and the one containing the elongate type, were treated in exactly the same way. They were killed at the same time

with the same fluid (in separate containers) and in the same water bath. Thence to 70 per cent alcohol the treatment continued identical. One hundred individuals from each sample of material were measured.

From these measurements the graphs shown in figure B were constructed in the same manner as the previous one, except that the curves of the separate samples were plotted separately on the same axis. The continuous line represents the individuals from one pig and the broken line those from the other. The mode of the first curve occurs at 1.3. If the ratios 1.2 and 1.4 be included with 1.3, it is found that 80 per cent came within these limits. Of the entire number of individuals in the lot only one showed a ratio of 1.6 and one as high as 1.7, while there were none with a higher ratio. The second curve reaches its highest point at 2.0, while 71 per cent of the entire number measured is included between 1.8 and 2.2. A number of individuals have ratios above 2.2, while one had a ratio as great as 2.7. Five individuals have ratios below 1.6, at which point the curves begin to overlap; but in practically every one of these individuals there were observed characters (which are discussed below) that made it quite evident that they were really of the type represented by the other curve.

Upon comparing figures A and B their likeness is very striking, the second being corroborative of the results shown by the first. That both are bimodal is evident. However, the low points, the point of demarcation of the two groups, do not occur at the same place; in the first it is at 1.4, while in the second it occurs at 1.6. Also the median of the first mode in figure A occurs at 1.2 while in figure B it is at 1.3, and the median of the second mode in figure A is at 1.7 while in figure B it occurs at 2.0; that is, in figure A the entire curve is shifted to the left, meaning that all ratios are decreased or that all individuals approach nearer to the spherical shape. This shifting is greatest in the case of the second mode. This in conjunction with the greatest breadth of the second curve in figure B is what would be expected if the premise in regard to the effect of fixation discussed above (page 251) is correct. Extra care was used in the fixation of the latter lot of material whereas in the former only the ordinary precautions were taken. At any rate these differences between the two groups do not detract from the evidence which they offer that there are two distinct types of balantidia parasitic in pigs.

The value of these curves in showing race or species differentiation is directly proportional to the extent to which any other factors which

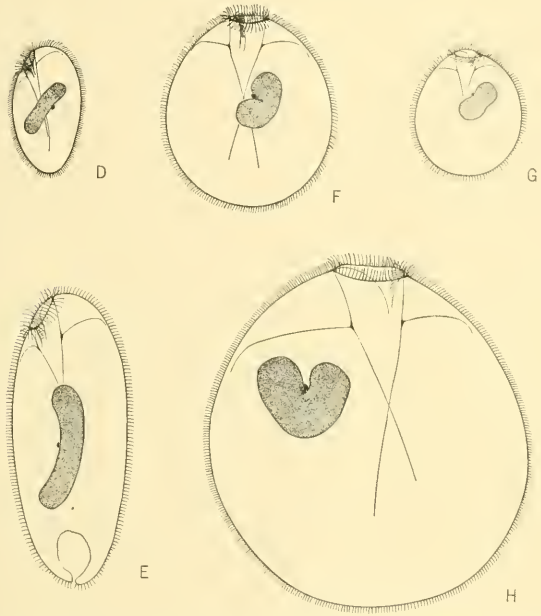
might produce a bimodal curve are non-operative. Factors involved in faulty technique were eliminated as far as possible. Over the effect of growth or age variation, however, the observer has no control. The possibility of these variations producing such curves as the one above is precluded by reference to the data from which the curves are constructed. Among the individual measurements recorded in Table I it will be noted that there are small individuals measuring $27 \times 50\mu$, and others measuring $42 \times 50\mu$; and that among the larger individuals, some measure $50 \times 120\mu$, and some, $90 \times 150\mu$. Further study of Table I shows that the two types are found among *all* sizes of individuals; consequently the variation of body proportions represented by the graphs is not correlated with variations of size, and probably not with the age or growth of the individuals.

That the variation could be accounted for by the occurrence of fission seems unlikely. Individuals might continue to elongate until binary fission occurred, and then by this process they might be shortened and the body proportion changed. Two considerations oppose this explanation. In figure A nearly equal numbers are of the respective types; in figure B each example contained almost exclusively one type of individual. In the former material very few dividing individuals were found, while in the latter not a single individual was seen in fission in either sample of material. But to accord with the above explanation one would expect to find many dividing forms among the elongate individuals represented by the broken line. In the second place, if this explanation were valid one would expect curves showing variation of body proportions to be continuous. Such is not the case, as is shown in figures A and B where the curve is bimodal due to a decided decrease of individuals having proportions intermediate between the two types.

The possibility of any effect from gametic variation, was eliminated by the study of conjugating forms, through which it was determined that isogamy was the rule. Likewise the possibility of influence of the quality of intestinal content of the host was eliminated by the frequent occurrence of both types in the same host. Other factors it would seem must be of minor importance and should give way for more positively corroborative evidence which may be found in correlated morphological difference.

Other specific characters.—In addition to the differences in relative lengths of the axes, one notes a distinct difference in the points of intersection, due to the variation in the shape of the types as pictured

in figures D, E, F, G, and H. The one form resembles very closely a hen's egg, the small end being anterior. In this case the longest diameter crosses posterior to the midpoint of the longitudinal axis. In the elongate type, usually the posterior end is as much drawn to



Figs. D–H. Camera lucida drawings of *Balantidium suis* sp. nov. (figs. D and E), and *Balantidium coli* (figs. F, G, and H), showing specific differences.

a point as is the anterior, and often more so. In these cases the longest diameter intersects the longitudinal axis at or anterior to its midpoint.

Coincident with the taking of measurements a careful search was made to detect other morphological differences which might occur between the two forms and be of aid in distinguishing one from the other. The earliest difference to be noted related to the macronucleus. The macronucleus in the ovoid type is relatively short, being approximately ⅓ the length of the entire organism. It is customarily bean-shaped in appearance, but may be almost straight or so sharply bent

at its middle as to form a short V (fig. I), and its width averages about 0.4 to 0.5 of its length. In the slender types the macronucleus is relatively long and slender, being approximately ½ of the entire length of the organism. It is ordinarily sausage-shaped, but it may also be in the form of a straight rod slightly enlarged toward the ends, or it may be so curved as to form an almost complete ring. In contrast to the form described above, the width in this case is about 0.2 to 0.3 of its length. Differences so great as these, viz., a length 2 to 3 times the breadth in one case and 4 to 5 times the breadth in the other are easily recognizable without actual measurement. These figures represent the average and do not mean that the limits of the two never overlap. This difference in nuclei serves as one of the easiest and surest ways of distinguishing the two types, for though the organism during locomotion may modify its proportions tremendously this does not noticeably affect the nucleus. It has been impossible to determine any difference between the micronuclei of the two types.

A very noticeable difference concerns the relative position of the cytostome. In the ovoid type the cytostome is almost, though never quite, terminal (see figs. F, G, and H). As mentioned previously, the anterior end is ordinarily drawn out to form a fairly decided point which lies within the area enclosed by the adoral cilia. In the slender type the cytostome is more laterally placed. The posterior limit of the right lip of the cytostome may extend ventrally to a point ⅕ of the length of the animal, in which case the dorsal portion of the adoral circlet of cilia may pass approximately through the terminal point of the body, but in normal form this point will never be within the adoral area. The parts which make up the adoral region of the animals show no fundamental differences except variation in the relative position of parts due to the lateral displacement of the cytostome. For example, the plane of demarcation between ectoplasm and endoplasm, which is approximately vertical to the long axis of the animal in the ovoid form, is at a decided angle, the ventral edge lying farther posterior in the elongate forms (see figs. D and E).

In the opinion of some authors the ventral displacement of the mouth is very significant (Delage and Hérouard, 1896; Minchin, 1912). These authors believe that the ventral displacement of the cytostome is progressive with evolution of the organism; i.e., that in the more primitive types the cytostome is terminal while in advanced groups it is successively displaced farther ventrally. Viewed in this light, the position of the mouth is here of considerable significance as

a specific character. Attempts to discriminate between the two forms on the basis of cytopyge or vacuoles were without result.

The specific differences just discussed have seemed to indicate a sufficient degree of separation of the two types to warrant the division of the ciliates of the genus *Balantidium* which occur in the pig into two distinct species. The description by Malmsten (1857), in conjunction with the figures (Malmsten, pl. 1, figs. 1–6) which he published, make it practically certain that the ovoid type is the one originally described by him and to which he gave the name *Paramoecium (?) coli.*

So far as I have been able to determine, the elongate species above described has never before been distinguished from *Balantidium coli.* For this new species I suggest the name *Balantidium suis.* As a summary of the specific characters discussed above I give the following description:

Balantidium suis sp. nov.—Body elongate; length approximately twice the breadth and varies from 35 to 120μ; breadth from 20 to 60μ; usually tapers more posteriorly, is blunter anteriorly, longest diameter transects longitudinal axis anterior to its midpoint; adoral region ventrally placed, cytostome $\frac{1}{5}$ of way posteriorly along ventral surface; nucleus rod or sausage-shaped, at least one-half the length of the entire organism, its width about one-fourth of its length; the species is parasitic in the pig.

The specific name, *Balantidium suis,* seemed fitting since it indicated the common host, *Sus scrofa.* Whether or not this species occurs in man it has not been possible to determine conclusively. A review of published case records of balantidiasis seems to show that it does not, but only a few of these records are accompanied by figures or descriptions of the organisms which are adequate for making positive discrimination. Fortunately, I have been able in two cases to make some direct observations.

Through the kindness of Mr. W. H. Barnes, of the Department of Pathology of the University of California, I was permitted to study sections of the human intestine which he had obtained at an autopsy following a fatal attack of balantidiasis. Imbedded in the serous and subserous layers were numerous balantidia. Measurements to show proportions were of little value under the conditions, for the form of each organism was largely determined by pressure, exerted by surrounding tissues. But from other characters, the type of nucleus especially, it was conclusively determined that the species there present was *Balantidium coli.* No individuals of *Balantidium suis* were found.

Measurements of balantidia as they occur free in the human intestine were made possible through the kindness of Dr. E. L. Walker, of the Hooper Institute of Medical Research, who loaned me several slides which he had prepared while in the Philippine Islands. This material had been stained with haematoxylin. Using the same precautions as in previous work, a total of 100 individuals was measured. The data were handled as before and the resulting graph is shown in figure C. In comparison with previous graphs it will be noted that this graph closely approaches coincidence with the curves representing *Balantidium coli*, for its mode is at 1.3 while the extreme portion of length and breadth is 1.5.

In addition to slides of human material, there was also loaned material from one pig and from one monkey. It is interesting that each showed a pure infection with *Balantidium coli*. The monkey (Monkey No. 10, Table I; Walker, 1913) had been experimentally infected by feeding it cysts from a pig, but not the pig from which the above-mentioned material was taken. Therefore this material yields no evidence regarding the validity of the specific differentiation nor the possibility of *Balantidium suis* becoming established in monkeys or in man.

There is no likelihood of confusing the new species, *Balantidium suis* with *Balantidium minutum* (Schaudinn, 1899). The differences are very marked. The body of the latter is oval, pointed anteriorly, more like *Balantidium coli*. The peristome reaches to the equatorial plane. There is but a single vacuole while there are two in each of the species considered here. The macronucleus is spherical, whereas it is elongate in both *Balantidium coli* and *Balantidium suis*.

MORPHOLOGY

Balantidium coli (Malmsten) and *Balantidium suis* sp nov. are ciliated protozoans, barely visible to the unaided eye, and are in a general way sac-shape (balantidium, *little bag*). Viewed through the microscope they appear grayish green in color. The homogeneity of the cell contents is broken by the presence of the nuclei, the contractile vacuoles, the food vacuoles, and sometimes by the presence of highly refractile bodies, the paramylum bodies. The entire surface of the body, except that of the oral plug, is covered with fine cilia. The cell contents are retained by a thin transparent pellicle which is protective

in function. The cytoplasm is distinctly differentiated into ectoplasm and endoplasm. The former constitutes a thin layer just underneath the pellicle and in it is situated the basal apparatus of the cilia. The layer of ectoplasm thickens greatly at the anterior end of the animal, to form, as it were, a matrix for the cytostome and its accessory apparatus. Within the ectoplasm, but not set off from it by a sharp line of demarcation, is the endoplasm. In the endoplasm are numerous food inclusions, often present in the form of starch or paramylum bodies. The macronucleus and the micronucleus are also within it, but seem to have no constant position in the cell. The macronucleus is either bean-shaped (as in *Balantidium coli*) or elongate and sausage-shaped (as in *Balantidium suis*). There are two contractile vacuoles, the larger being situated anteriorly and the smaller posteriorly. They lie closely beneath or may be entirely surrounded by ectoplasm, thus belonging really within that layer.

So far as can be determined the animals show no modification with respect to a substratum, yet the lateral and posterior displacement of the cytostome has lead to the designation of that side, toward which displacement occurs, as ventral, and the opposite surface as dorsal. This terminology is very nearly universal in the literature on *Balantidium*, and the correlated terms of right and left are used in the original description of the family Bursaridae (Stein, 1867) and the genus *Balantidium* (Claparède and Lachmann, 1858). The dorsal side may be somewhat more convex than the ventral; this occurs not infrequently in *Balantidium suis*, though, due to the plasticity of the organism, this is by no means constant. No part of the body is differentiated for skeletal purposes. The anal aperture or cytopyge is at the posterior tip and may be present as an actual aperture or only as an extreme thinning of the ectoplasm and pellicle at this point. In connection with it there is usually a rectal vacuole which serves as a storage reservoir for solid waste awaiting extrusion.

Ectoplasmic Structures

Pellicle.—The entire body is covered by an extremely thin but resistant pellicle (*pel.*, figs. I and L). The pellicle seems to be somewhat thickened, as shown by a higher degree of refractibility, along the margin of the lips of the cytostome where it turns in to form the lining of the oesophagus and the groove in which the oral cilia are set; otherwise, it is nowhere noticeably specialized. It shows alternating

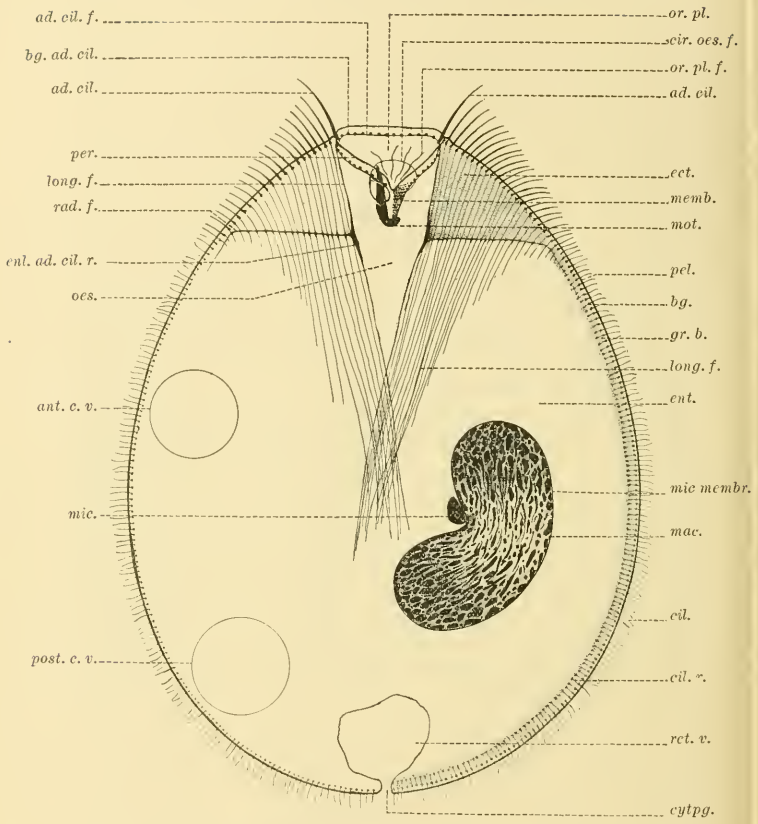


Fig. I. *Balantidium coli.* Ventral view showing principal structures. The adoral cilia, with exception of one at either side, are indicated by basal granules only. The adoral membranelles are also represented solely by the basal apparatus. Ectoplasm is shaded, and ciliary rootlets are shown on one side only. × 1250. *ad. cil.*, adoral cilia; *ad. cil. f.*, adoral ciliary fiber; *ad. memb.*, adoral membranelles; *ant. c. v.*, anterior contractile vacuole; *bg.*, basal granule; *bg. ad. cil.*, basal granules of adoral cilia; *cil.*, cilia; *cil. r.*, ciliary rootlet; *cir. oes. f.*, circumoesophageal fiber; *cytpg.*, cytopyge; *ect.*, ectoplasm; *enl. ad. cil. r.*, enlargement of adoral ciliary rootlet; *end.*, endoplasm; *gr. b.*, granular band of ectoplasm; *long. f.*, longitudinal fibers; *mac.*, macronucleus; *mic.*, micronucleus; *mot.*, motorium; *nuc. memb.*, nuclear membrane; *ocs.*, oesophagus; *or. pl.*, oral plug; *or. pl. f.*, oral plug fibers; *pel.*, pellicle; *per.*, margin of peristome; *post. c. v.*, posterior contractile vacuole; *rad. f.*, radial fiber; *rct. v.*, rectal vacuole.

ridges and grooves, the cilia passing through the bottom of the latter, but this condition is not due to longitudinal thickenings in the pellicle itself, but to the fact that it is closely applied to the ectoplasm which is thus furrowed. It can often be separated in "blisters" from the ectoplasm by tannic acid or weak alcohol, and when thus removed it shows regular longitudinal rows of perforations through which the cilia pass out from the ectoplasm. In this condition its transparency is very evident. The pellicle is not extremely impervious. For instance, when the active animals are introduced into normal salt

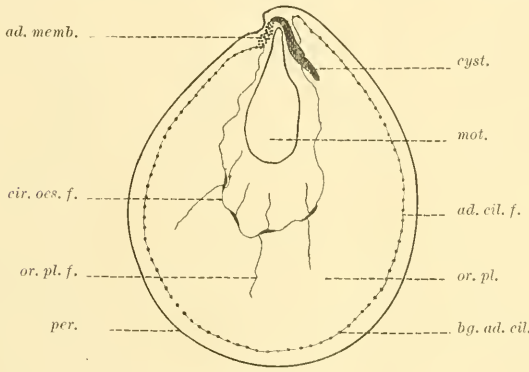


Fig. J. The neuromotor apparatus of the adoral region, anterior view. × 2000. *ad. cil. f.*, adoral ciliary fiber; *ad. memb.*, adoral membranelles; *bg. ad. cil.*, basal granule of adoral cilia; *cir. oes. f.*, circumoesophageal fiber; *cytst.*, cytostome; *mot.*, motorium; *or. pl.*, oral plug; *or. pl. f.*, oral plug fibers; *per.*, margin of peristome.

solution plasmolysis takes place very quickly, and they present a grotesque appearance as they swim about with several huge depressions in their surfaces due to the shrinkage. *Intra vitam* stains such as neutral red and Janus green also penetrate very quickly. Resistance to pressure and mechanical change, however, is very marked, and is due to its tenacity and flexibility, both of which qualities are shown when the animal forces itself through an opening much smaller than the normal diameter of its body (fig. K) and also by the extreme flattening which it withstands under the increasing pressure of the coverglass when evaporation of the preparation is allowed. As previously mentioned, these qualities indicate that the pellicle is protective and retentive in function rather than supportive or skeletal.

These organisms show remarkable mobility when observed under conditions as nearly normal as possible. I have attempted to depict something of this plasticity in figure K. As they travel amid the débris in the intestinal contents, which has been removed with them, a tendency to penetrate is much more noticeable than any avoiding reaction. Instead of reversing the ciliary action, backing away and taking a new direction as would paramaecia, the balantidia, when they come in contact with a solid object, rather apply themselves to the surface, round up, and seem to roll along it. After a moment of such slow contortion, they may swim away in a new direction,

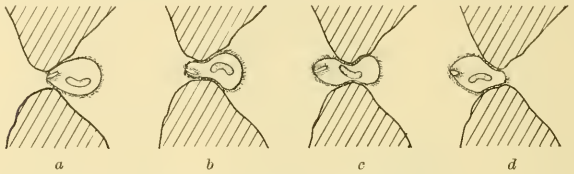


Fig. K. Diagrammatic illustration of the plasticity of the organism, resulting in ability to pass through remarkably small openings.

determined by the direction of the anterior end. They avail themselves of the slightest opportunity to force their way through or between any obstacles. The anterior end, especially the thickened ectoplasmic portion, becomes at such times decidedly elongate and conical (fig. K, *b*). The cilia of this region beat spirally producing a boring action as this anterior tip is protruded into any slight opening. This action has in many instances been observed to cause two obstacles, either of which was larger than the organism itself, to separate sufficiently to allow it to pass between. The aperture need not be one-half of the diameter of the animal for the latter will constrict (fig. K, *c*) and the fluid contents flow through anteriorly as it progresses, resembling the process of putting a bag of beans through a small hole in a board. Throughout observations of the activity of these organisms, one is impressed with their fitness for penetrating the mucous lining of the intestine and the underlying tissues. Its thigmotropic response, its boring action, and its extreme plasticity, all seem to be adaptations for the function of penetration.

Ectoplasm.—Immediately underneath the pellicle, the cytoplasm is differentiated to form the ectoplasm (*cct.*, fig. I; pl. 27, figs. 1–7). The ectoplasmic layer, except at the anterior end, does not exceed two

microns in thickness. The anterior end of the animal, that is, all anterior to a transverse plane which would transect the body at a point 1/6 to 1/5 of the way to the posterior end, is composed entirely of ectoplasm. In this cone-shaped area is located the cytostome and a large part of the neuromotor apparatus. The protoplasm of this region seems to be homogeneously granular in fundamental structure. It stains very deeply with haematoxylin; so deeply in fact, that in differentiation it is necessary to destain other parts of the body almost completely before this part reaches a degree of transparency suitable for study. With Mallory's connective tissue stain this region also stains very densely, taking on both the brilliant red and the deep blue elements of the stain, in different structures, as will be explained below. This extensive thickening of the ectoplasm at the anterior end is clearly shown in the figures by Leuckart (1861) and has been noted by nearly all who have studied the animal more recently, but I have failed to find any discussion of its significance. This same phenomenon occurs in the Ophryoscolecidae, as pointed out by Sharp (1914) in his work on *Diplodinium* and by Braune (1913) in *Ophryoscolex*. In these cases the change seems to be correlated with the high degree of activity and specialization of the anterior end of the animal; in *Diplodinium* in connection with its selective feeding, and in *Balantidium* in connection with both feeding and activity of this entire region in penetrating the mucosa of the intestine. The centering of the neuromotor apparatus in this region gives additional evidence in regard to this question which will be discussed further in connection with the description of that apparatus.

Throughout the entire investigation of the minute structure of this animal, I have been unable to demonstrate the presence of any definite plane of demarcation between endoplasm and ectoplasm, such as the "ectoplasmic boundary layer" described by Sharp (1914) in *Diplodinium,* and shown in plate IV, figure 3 of his paper. Many of the fixed preparations used in the search for such a layer were sections of the animal, treated as nearly as possible according to the technique used by him and stained, as were his preparations, with Mallory's connective tissue stain. So far as it was possible for me to determine, any sharp boundary line between ecto- and endoplasm is lacking. On the contrary, they merge into one another and only in a general way can it be said where one terminates and the other begins.

Prowazek (1913, fig. 2) describes, in *Balantidium coli,* "ein Art von Zwischenmembran" which appears as wavy lines in optical sec-

tion. According to his interpretation this "Querlinie" separates the protoplasm of the cell body into two regions, the "apical zone," which I have described above as a thickening of the ectoplasm, and the rest of the cell protoplasm. The extent of this "Zwischenmembran" he does not note, but his text figures (1 and 2) do not show it as extending quite to the pellicle, but instead as stopping short of the pellicle at a distance about equivalent to the thickness of the ectoplasm at that point. This is significant in my interpretation of this region, namely, that what appears as a continuous line or plane when viewed from the side is in reality, as shown in cross-sections (pl. 27, figs. 6 and 7), a set of diverging fibers. These fibers take origin from dark-staining

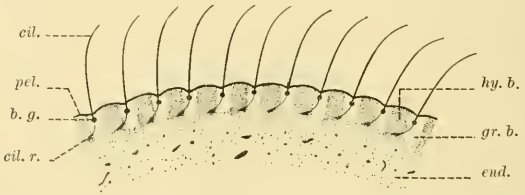


Fig. L. Portion of the peripheral region of a cross-section of *Balantidium coli*, showing the structure of the ectoplasm and arrangement of cilia, somewhat diagrammatic. × 1500. *b. g.*, basal granule; *cil.*, body-cilia; *cil. r.*, ciliary rootlet; *end.*, endoplasm; *gr. b.*, granular band of ectoplasm; *hy. b.*, hyaline band of ectoplasm; *pel.*, pellicle.

enlargements on the longitudinal fibers in the wall of the gullet and, diverging, pass peripherally until they turn posteriorly at the very inner edge of the thin layer of ectoplasm which covers the remainder of the body (pl. 28, figs. 9–12). Even in lateral view careful focusing will often show that the apparent "membrane" is really discontinuous, showing breaks and irregularities as one focuses on different levels and hence can not be considered as a true membrane. The arrangement of these fibers will be described more exactly under the discussion of the neuromotor apparatus.

The ectoplasm which constitutes a layer less than 3 microns in thickness around the remainder of the periphery of the cell shows a definite and somewhat complex structure. In tangential sections of the surface, which are so thin that they do not include much of the underlying endoplasm, one detects alternating light and dark longitudinal spiral bands (*gr.b.*, *hy.b.*, fig. N). These bands are parallel to the rows of cilia and very nearly equal in width. In cross-sections (fig. L) they are seen to extend nearly, if not quite the full depth of

the ectoplasm. As one follows these bands (or "stripes," as they are named by Johnson (1893, in his work on *Stentor*) anteriorly they seem to lose their distinctness when they become continuous with the apical cone. In some individuals, however, one can follow them some distance into this cone, but never is there the same degree of differentiation of the two areas in this region.

In the living animals, which are often quite opaque due to inclusions, it is nearly impossible to distinguish these longitudinal light and dark bands. With neutral red the dark or granular band stains faintly. With the haematoxylin stains used in thin sections of fixed material the dark band seemed to be finely granular in fundamental structure. The granularity in this case must be determined largely by the general appearance and stainability, for the individual granules are so small as to defy identification. There is no indication, however, of alveolar structure, so that the term *granular* is probably the more applicable and will be used to distinguish this from the light band. The latter takes only faintly the stains used and seems hyaline in structure. The granular bands lie directly beneath the ridges in the cuticle which occur between the rows of cilia. Or more correctly, the ridges on the surface of the animal are produced by the projection of these granular bands outwardly beyond the hyaline bands. These latter are directly beneath the grooves of the surface where the cilia pass through the cuticle and attach with the basal granules which lie in longitudinal rows; a single row in each hyaline band. The ciliary rootlets (*cil. r.*, figs. I and L) extending in from the basal granules proceed diagonally inward and pass into the interior margin of the granular band.

Stein as early as 1876 pointed out these alternating dark and light stripes in *Stentor*. To the granular and bright stripes, Bütschli (1889) gave the names "Riffenstreifen" and "Zwischenstreifen," respectively. Johnson (1893) gives a careful description of these bands as they occur in *Stentor coeruleus*. He notes that they vary greatly in width, and this is true in *Balantidium;* but both bands are much narrower than in *Stentor,* the combined width of the two not exceeding two microns. In *Stentor coeruleus* Johnson (1893) gives the width of the granular band as 22μ and that of the bright band as 7μ, these measurements being taken just under the adoral zone. It is interesting to note that in *Balantidium coli* the granular band is also slightly wider than the bright band. These bands become narrower from the region of the greatest circumference of the animals toward

each end, which is comparable with the arrangement in *Stentor,* though in the latter the narrowing must necessarily take place in the posterior direction only. The above author mentions the branching of stripes, but this does not occur in *Balantidium* so far as I have been able to determine. As the bands pass posteriorly, however, they become less distinctly differentiated and are hard to follow, and it might be that further study with more intensive stains would reveal a union in the region of convergence at the posterior end. More recent work has added to the number of Heterotricha that show this sort of differentiation of ectoplasm. Maier (1903) shows the striped nature of this layer in *Prorodon* and *Spirostomum,* while Neresheimer (1903) confirms the structure found by Johnson (1893) in *Stentor.* Schuberg (1887) also indicates a comparable plan of structure in *Bursaria.* The granular ridges of ectoplasm between the furrows in which the anal cirri are situated in *Euplotes patella,* discovered by Yocom (1918), may be comparable with the bands which occur in Heterotricha.

Cilia.—The entire surface of *Balantidium coli,* with the exception of the oral plug, is thickly beset with cilia (*cil., ador. c.,* fig. 1). These are of two kinds, viz., those which make up the adoral row of cilia and which measure from 8 to 12μ in length, and those covering the body, which vary from 4 to 6μ. Those covering the apical cone form an intergradation between the two. On this surface the cilia which occur immediately posterior to the adoral row are only slightly shorter and slightly more slender than the adoral cilia themselves. Passing posteriorly they gradually become shorter and less cirrus-like until they reach the base of the apical cone. Thence posteriorly they retain the uniform size.

The body cilia are comparatively short and very slender. So small are they in fact that to observe a single one is nearly impossible. In slides prepared by the usual methods no stain remains in the cilia if destaining is carried sufficiently far to differentiate other structures. Iodine (Weigert's solution) gives a fairly satisfactory stain for temporary mounts. The arrangement of cilia may be determined most readily by using a heavier stain and then observing the distribution of basal granules. Neutral red proves very satisfactory for this purpose. The cilia occur in longitudinal, slightly spiral rows, following the grooves between the ridges in the pellicle. These rows originate immediately posterior to the groove in which the adoral cilia are set and for a very short distance pass almost meridionally; very soon,

however, they turn toward the left, that is, in a counter-clockwise direction when the animal is viewed from a point exactly in front. They continue their spiral direction until almost to the posterior end when they again follow a meridional path to their termination. In passing the entire length of the body any single row of cilia twists to the left approximately 120°, or one-third the entire circumference. Whether some rows terminate or become continuous with contiguous rows before reaching the posterior tip of the animal, I have been unable to determine, for both basal granules and granular bands become very indistinct in this region even in the best preparations. The number of rows was counted with difficulty in several cross-sections from the equatorial regions of different animals, and it was found to vary from about 60 in small individuals to 120 in larger ones. No correlation between the variation in the number of rows of cilia and the species of the animal could be determined, but this is possibly due to the limitation of observation.

Basal apparatus.—The cilia perforate the pellicle and attach to the basal granules which lie immediately underneath (fig. L). The latter are small and apparently spherical or oval. They stain very deeply black or blue with haematoxylin. In the living animal they are readily emphasized by the use of neutral red, and less so by Janus green. In preparations stained with Mallory's connective tissue stain these granules show brilliantly red with the acid fuchsin, as do the other parts of the neuromotor apparatus and also the micronucleus. Longitudinally the granules are so closely placed that it is impossible to observe whether they are actually connected by a fibre. Cross-sections show that the cilia of one row have no transverse connection by any sort of stainable fiber with those of the next row. The rows of basal granules lie close beneath the depression in the pellicle in the hyaline or bright band of the ectoplasm. A ciliary rootlet (*cil. r.*, figs. I and L) extends from each basal granule centrally toward the endoplasm. It does not proceed in an exact radial line but rather diagonally toward the right until it enters the granular band near the inner surface of the latter. The ciliary rootlets in some cross-sections appear to have an exactly radial direction. In such cases, however, the granular band is somewhat diagonal in the opposite direction. This variation is probably produced either by torsion of the animal or by the direction of the effective beat of the cilia at the instant of fixation. The diagonal direction of the rootlets is readily detected in tangential sections. In focusing down through such a sec-

tion, the rootlet is invariably seen to run from the basal granule toward the observer's right into the contiguous granular band on that side. Thus, to avoid any confusion that might arise from the terms right and left, in a cross-section of the animal viewed from the anterior surface (such a view is shown in fig. L) the ciliary rootlets swerve in the counter-clockwise direction and enter the granular band lying immediately in that direction.

As the rootlet enters into the granular band it apparently enlarges thus forming a secondary basal granule. In some cases this may stain even more deeply than the basal granule itself, and appear as a definite body somewhat elongated in the direction of the circumference of the animal. It was thought at first that this might be the cross-section of a longitudinal fiber or myoneme. But the study of numerous tangential sections has failed to show the presence of any longitudinal fiber within the granular band. The stainability varies greatly and in preparations which have stained lightly the ciliary rootlets appear to fray out and merge into the granular band, while still retaining deeper color than the rest. Which interpretation is correct it is difficult to say, but the latter seems the more probable, especially in view of certain relations with the neuromotor apparatus which will be discussed later.

Pütter (1903) reproduces a figure from Studnicka (1899) showing in a schematic way five types of attachment of the cilia with their basal apparatus. Of these, two, at least, represent cases in which two basal granules or a diplosome are present. Saguchi (1917) in his studies on ciliated cells of Metazoa says in part regarding the basal granules of certain ciliated cells from amphibian larvae, "With favorable staining the basal corpuscles appear as diplosome or dumbbell shaped granules. One of these is situated at the upper the other at the lower border of the cuticle." In *Balantidium coli* the arrangement with respect to protoplasmic layers is quite different, though the cilia seem to follow somewhat the same plan of structure even to the presence of rootlets.

The most fruitful comparison may be made with the basal apparatus of cilia in *Isotricha prostoma* as described and pictured by Braune (1913). In this organism he describes diplosomic structure of the basal apparatus, in which the basal granule lies directly beneath the pellicle. The cilia, however, extend beyond the basal granule into the underlying layer—the "Zwischenschicht" of Eberlein, and terminate in the "Grenzschicht" with a second granule, which upon

maceration remains attached to the basal end of the cilium. So the basal apparatus in *Balantidium coli* even to the relative location of the basal granules is almost identical with that in *Isotricha prostoma*. In *Balantidium*, however, as mentioned above, the "Grenzschicht" seems to be lacking. The comparison becomes more significant in view of the fact that both ciliates are parasitic in the digestive tract of mammals, and both are in much the same state with reference to the degree of specialization and degeneration correlated with habits of the parasitic mode of life. So that in general morphology they seem to be more alike, though they are in separate orders, than do *Diplodinium caudatum* and *Balantidium coli*, which are in separate suborders only.

Ciliary Movements.

Locomotion is the chief function of the cilia except for those of the adoral zone. The balantidia swim in approximately a straight line and not in a spiral course as do paramoecia. They do, however, rotate on their axis as they progress. This rotation is generally from left to right, that is, in a counter-clockwise direction when viewed from a point in front of the animal. A few instances of reversal of the direction have been seen, but it is not at all common. The direction of rotation, i.e., from left to right, seemed at first inexplicable, since this was not compatible with the direction of the rows of cilia. The rows of cilia, as described above, are comparable to the threads of a left-hand screw. In order to penetrate, such a screw must be turned in a clockwise direction (when viewed from the point, not from the head). Such, also, is the direction of rotation of balantidia which one would expect to find if the arrangement of the cilia were the controlling factor, but the rotation is in the reverse direction. In the further study of the problem, I fortunately obtained some very thin tangential sections of animals on which the fixative had acted so quickly that the cilia were stopped instantly and left in the relative positions assumed in normal ciliary action.

Figure N is a camera lucida drawing of such a section. By analysis of the position of the cilia on this and other like sections, it was possible to determine the complete cycle of a single cilium. This cycle is diagrammatically represented in figures M and O. Figure N includes about two and one-half cycles of action as represented by the waves. The dark portions are produced by the prostrate position of the cilia at the termination of the effective stroke. The lighter

areas between are due to the fact that the cilia are recovering their vertical position and hence are viewed very nearly endwise. The position of consecutive cilia in any single row, from one point in a wave to a similar point in a following wave will fairly represent the successive positions taken by a single cilium in making one complete cycle. Figure M was made in this way. The arrow represents the long axis of the animal. From this diagram it is seen that the cilium at the end of the effective stroke lies rather close to the surface of the body and not along the row but decidedly to the left from it. In

Fig. M

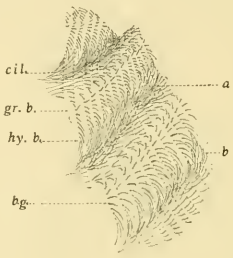


Fig. N

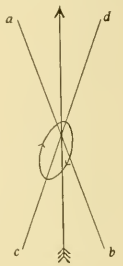


Fig. O

Fig. M. Diagrammatic representation of the successive steps in one complete beat of a cilium of *Balantidium coli*. It also illustrates the positions of the respective cilia of a single row between the points *a* and *b* in fig. M, at which points the cilia are in a prostrate position at the end of their effective stroke. The arrow indicates the long axis of the animal.

Fig. N. Tangential section of *Balantidium coli*. The cilia still retain respective positions which they had in the normal swimming movements of the organism. × 1500.

Fig. O. Diagram illustrating the effect of the ciliary action in the rotation of the organism. The arrow represents the long axis of the animal; *ab.*, the direction of the rows of cilia; *cd.*, the direction of effective stroke of a cilium attached at the point of intersection of the three lines; the ellipse is described by the tip of the cilium.

recovery it straightens up and passes anteriorly, thence to the right, crossing the row, of which it is a part, at right angles. At this point the cilium leans anteriorly only slightly from the vertical. The effective beat is produced by the quick stroke of the cilium posteriorly and to the left, and continues until the cilium has crossed the row again and lies close to the surface and extends to the left as represented by the position of the last cilium shown in figure M. According to the classification given by Pütter (1903), the movement of the cilia of *Balantidium* would be called infundibular. As will be noted from

figure O, the funnel described by the complete beat of the cilium is somewhat irregular, the rim outlined by the tip of the cilium is elliptical, and the base of the cilium, i.e., the neck of the funnel, is not central but is situated below the anterior focus of the ellipse. The ellipse described by the tip of the cilium lies in a plane which is not parallel to the surface of the animal, but which approaches it much more closely posteriorly. It was possible to corroborate the action of the cilia in observations on the living material. The cilia of the apical cone are somewhat larger than the other cilia of the body, and are closely coördinated in their action. In balantidia which were allowed to cool until action had slackened considerably, these cilia were observed to make their effective stroke at a decided angle to the rows of cilia. For instance, in the boring action in connection with the process of penetration described above, these cilia will beat in an almost exactly transverse direction, always from right to left. In further corroboration of this interpretation of the movements of the cilia is the fact that it explains the rotation of the animal during progression. It will be seen from figure O that the effective stroke of any single cilium will be in the direction *cd*. This line crosses both the arrow, representing the long axis of the animal, and the line *ab*, representing the rows of cilia, making an angle of approximately 20° with the latter. That is, the line *cd* forms an angle with the arrow on one side about equal to the angle on the opposite side made by the line *ab*. It clearly follows that if the effective stroke is in the direction *cd* then rotation will be from left to right and not vice versa as would be the case if the cilia beat in the direction of the rows in which they are arranged. The structure of the basal apparatus is significant in view of the direction of the effective stroke of the cilia, viz., posteriorly and to the left. The cross-section shown in figure L is viewed anteriorly and shows that the ciliary rootlet from each basal granule passes to the right and enters the granular band on that side of the hyaline band in which the basal granule lies. Without giving to the ciliary rootlets any motor or skeletal function, it still seems logical that they conform in a general way to the axis of the cilium, for the latter during the greater part of its movement inclines to the posterior and left of the row of which it is a constituent.

The peristome (*per.* fig. J) may be said to comprise all that part of the organism which lies within the row of adoral cilia. In the active animal it is variable in shape. At times it is almost round while at other times it may become a mere slit or groove. In what

seems to be its more normal proportions, however, it is approximately pear-shaped with the stem end of the pear directed ventrally. In *Balantidium coli* the adoral zone commonly includes the most anterior point or apex of the animal—at least its dorsal margin passes through this point. In *Balantidium suis*, the anterior tip of the animal lies wholly outside of this zone, the latter having migrated too far ventrally to include it.

The *cytostome* (*cytst.*, fig. J) does not occupy the whole interior of the peristome, but is situated at its ventral end. There is some indication that this aperture may be completely closed by the oral plug (*or. pl.*, fig. I) which comprises the rest of the peristome within the adoral row of cilia. This oral plug bears no cilia. It is exceedingly mobile, adapting itself readily to the almost constantly changing shape of the apical cone. It lies dorsal to the cytostome and is not exactly bilaterally symmetrical since it is pushed somewhat to the left to make room for the oesophagus. It extends inward, thinning as it does so, until it terminates about the beginning of the endoplasm. It is ectoplasmic, but very finely granular as compared with the rest of the ectoplasm. Mallory's connective tissue stain ordinarily gives it a decidedly bluish tinge with slight spots of red only where there are certain neuromotor fibers. Its action in feeding is very hard to follow, but its high degree of mobility impresses one when watching the activities of the organism, and may be demonstrated with fixed material by its extreme protrusion (pl. 28, fig. 14). In addition to this, the fact that it is intimately connected with the neuromotor apparatus would indicate that it functions in selective feeding. The oesophagus (*oes.*, fig. I; pl. 27, figs. 4–8) beginning at the cytostome, passes inwardly, not quite radially but swerving slightly to the right. It may be followed definitely through the ectoplasm and for a very short way into the endoplasm where it ends blindly. So far as I have been able to determine, it is a uniform tube-like opening without evident enlargements or constrictions. Prowazek (1913), however, gives in part the following description, ". . . . es sehnt sich jedoch nicht direct trichterformig in die Tiefe, da man von der drei scharfe Konturen noch nachweisen kann (fig. 1)." While studying living forms stained with neutral red, I have often observed specimens in the exact position of the one shown by Prowazek (1913, p. 7, text fig. 1). The lines shown by him were easily recognizable, deeply stained with the neutral red, but I could interpret them only in the following way. The most anterior line which he shows seems to be

clearly the ventral lip of the peristome; the second line was identical with the deep staining motorium (fig. I) in the forms which I studied, and the most posterior line seemed identical with the ring of enlargements on the rootlets of the adoral cilia which lie close about the oesophagus. The margin of the peristome is slightly raised, forming a ridge or lip. This ridge is most pronounced from the midventral point, dorsal along the right margin of the cytostome, but as it proceeds around it becomes rather inconspicuous and wholly disappears on the left side. Thus the cytostome has the appearance of opening under a ledge, the ledge formed by the lip of the right side.

The peristome is delimited by an almost complete spiral circlet of cilia, the adoral cilia (*ad. cil.*, fig. I). The exact point of origin of the row is hard to determine but it is approximately at the ventral edge of the peristome, i.e., on the ventral lip of the cytostome. From here it proceeds in a sort of groove, along the right margin, around the dorsal margin, and down the left margin of the peristome. A short distance in advance of the ventral point, the row of adoral cilia turns into the cytostome and continues in a spiral course down the oesophagus; entering at the left dorsal side and ending in the ventral wall about halfway down. Thus these cilia in their entire course make one complete left-hand spiral, beginning on the ventral lip, passing around the peristome and down the oesophagus, terminating in its ventral wall.

Over the lip of the cytostome between the point where the adoral cilia turn into the oesophagus and the midventral point where this row of cilia has its origin, the longitudinal rows of body cilia turn in. Each of these rows, of which there are ten or twelve altogether, continues down the oesophagus until it meets the row of adoral cilia. Since this latter enters spirally, there is a ciliated patch on the ventral wall of the funnel-shaped oesophagus which is roughly the shape of a right triangle, the base of which is the lip of the cytostome. The hypotenuse is the row of adoral cilia which makes an acute angle with the longitudinal row of body cilia which enters in the mid line and which represents the third side of the triangle.

The adoral cilia, except where they lie within the oesophagus, are completely separate each from the other. This is easily verified by watching their action especially in a disintegrating animal, or one cooled to slow up ciliary movement, under which condition coördination is frequently interrupted, and a single cilium will be seen acting independently of its neighbor. Additional evidence is to be found in

the individuality of each basal granule. There is no evidence of fusion of granules, though adjoining granules are connected by a neuromotor fiber. However, in the triangular area mentioned above, there is considerable evidence that the cilia are united to form membranelles. It is very difficult to watch the action of these cilia, and the above conclusion was reached largely through a study of fixed material. In cross-sections (pl. 27, fig. 4) the cilia of the region seem to be very close together and quite regularly to be connected by, or to form, a sheet of almost transparent substance. The regularity of this occurrence would lead one to believe that it is the normal structure and not due to entanglement of the cilia in foreign matter. In addition, the basal granules of this region (pl. 27, figs. 4 and 5) do not stand out separately, but are so closely packed that they give the appearance of a single deeply stained mass. I have been unable to distinguish separate granules in the inner portion of the area and it seems likely that actual fusion of the granules may have occurred. The basal apparatus of this region is so densely packed and takes stain so readily that in certain views it may easily be mistaken for the motorium. As a result, it seems plausible to interpret this area as the basal apparatus of membranelles which lie in a plane transverse to the axis of the oesophagus. If this region be interpreted as a primitive oral groove or cytostome, a forerunner of such an elaborate arrangement as occurs in *Euplotes patella* (Yocum, 1918) or in *Stentor*, then comparison is very significant, for in the latter two the membranelles also run transversely in the cytostome. The only difference, then, between these organisms and *Balantidium* in this regard would be the difference in shape and extent of the area.

The adoral cilia (*ad. cil.*, fig. I) are approximately double the length of the body cilia, i.e., from 6μ to 8μ in length or, in the very large individuals, they may reach a maximum length of 10μ. In fundamental structure they are like the body cilia, but the relative position of parts is somewhat different. Immediately beneath the pellicle each cilium bears a basal granule and from this a fiber continues inward which passes between the adoral plug and the surrounding ectoplasm. The sum of all the fibers from adoral cilia marks off very distinctly the conical cytostomal region from the surrounding ectoplasm. They seem to constitute the only partition between the protoplasm of the two areas, for I have not been able to detect any membrane in this region making a complete separation of the adoral region from the rest of the apical cone. Where the fibers pass from

the ectoplasm of this cone into the endoplasm, each bears an enlargement which stains very deeply. This enlargement is undoubtedly homologous with the inner enlargements of the basal apparatus of the body cilia for it bears the same relation to the basal granule and the cilium. It is farther removed from the basal granule but it maintains exactly the same relation to the ectoplasm and endoplasm, that is to say, in either case this enlargement lies in the plane between the two. From each enlargement a radial fiber extends laterally along the base of the apical cone to the periphery of the cell. The portion of the ciliary rootlet which continues inward from this enlargement is very distinct and may often be traced almost to the posterior end of the animal (fig. I). In no instance has it been possible to demonstrate any attachment or connection of the posterior ends of these rootlets. They often cross near the center of the organism and then fade out, becoming indistinguishable in the endoplasm. For a short distance inward from the oesophagus they are so closely placed dorsally and laterally that they form a kind of wall, but ventrally they are less numerous and likely to be much shorter, so that the pseudo-wall is not complete. It is sufficient, however, to direct the food for some distance into the endoplasm. There is no such high degree of differentiation here as Sharp (1914) found in the oesophagus of *Diplodinium ecaudatum.*

The homology of the adoral and body cilia is evidenced by the complete series of gradations from the one to the other which exist in the cilia of the apical cone (fig. I). The cilia which are proximal to the adoral cilia are almost identical with them. They are slightly smaller, but each has the basal granule beneath the pellicle, a fiber connecting this with an enlargement at the plane of differentiation of ectoplasm from endoplasm, and the rootlet extending posteriorly. The inner enlargement is much smaller and the ciliary rootlet shorter than those of the adoral cilia. These enlargements are connected with the enlargements of the adoral cilia by the radial fibers mentioned above. Passing posteriorly the cilia become progressively smaller, the basal granule and the enlargement closer together (due to the approach of the transverse plane of demarcation between ectoplasm and endoplasm to the pellicle), and the ciliary rootlets become progressively shorter and less distinct, until the cilia of the marginal area of the apical cone become identical with the body cilia. This apical cone then shows us a complete gradation of differentiation between the body cilia and the adoral cilia, and proves their homology.

Feeding is the chief function of the adoral cilia. They are closely coördinated in action, as is shown by the fact that in degenerating individuals they will beat in unison after other cilia beat only erratically or have ceased entirely. Coördination is most pronounced in the cilia of the left lip. The action of the adoral cilia produces a strong eddy in the surrounding medium with the vortex of the eddy within the peristome. In the above description it was noted that the right lip projected slightly forming a ledge or bank. The current produced by the cilia strikes against this ledge and solid objects are deflected into the underlying cytostome. The natural rotation of the animal on its longitudinal axis as described above aids in this process, for the right lip thus acts as the blade of an auger. The food particles pass down the oesophagus and collect at the inner end in a sort of droplet. After several bits have been collected, the droplet begins its circulation in the endoplasm as a food vacuole.

The closure of the cytostome in all probability is effected by the *oral plug*. The latter is very mobile and contains fibers of the neuromotor apparatus. It has frequently been seen to project anteriorly in a knoblike protrusion (pl. 28, fig. 14). This same phenomenon was observed by Wising (1871). In view of its situation, its sensitivity, and its mobility, it seems plausible to interpret it as an oral plug with essentially the same function as the oral disk of *Diplodinium ecaudatum* (Sharp, 1914).

The discharge of indigestible portions of the food takes place at a constant point at the posterior end of the animal, the cytopyge (*cyt.*, fig 1). It is an opening, however, only at the time of discharge. At other times there is only a thinning of the cortical layer which can be identified with comparative ease in fixed material. In the living organism the process of defecation was frequently observed. The undigested particles become segregated at the extreme posterior end in a sort of vacuole, the rectal vacuole (*rct. v.*, fig. 1; pl. 28, fig. 13). After this vacuole has become of considerable size (often filling one-third of the cell in degenerating forms) the pellicle over the cytopyge ruptures and the contents are discharged. The pellicle quickly forms again closing the aperture, collection of indigestible particles in the rectal vacuole continues, and the process is repeated.

The *contractile vacuoles* (*post. c. v.*, *ant. c. v.*, fig. 1) are two in number, usually situated on the dorsal side, one anteriorly, well up toward the apical cone, the other in the posterior one-third of the organism. There is a great deal of variation in their location in different animals, though in the individual their situation seems to be

very constant, at least throughout the limit of possible observation. These vacuoles seem clearly to originate within the ectoplasm. When fully distended they encroach far upon the region of the endoplasm and it becomes impossible to tell whether or not they are entirely surrounded by ectoplasm; but from their origin such might be suspected to be the case. Also such is the case in many related forms, for example, *Diplodinium ecaudatum* (Sharp, 1914) and *Euplotes patella* (Yocom, 1918).

The pulsation of the vacuoles was observed in several instances for a long period of time. The rate of pulsation varies considerably, occurring as rapidly as once in every thirty seconds under some conditions, while under others a complete cycle from discharge to discharge occupies a period of five minutes. In degeneration the pulsation is likely to be very much retarded or may cease entirely, the vacuoles becoming enormously distended and breaking together thus forming one large vacuole occupying fully one-half of the interior of the organism. Following this the animal ruptures and disintegrates. The observation of a considerable number of normal individuals has shown the usual cycle to be as follows. At two points in the ectoplasm small droplets of clear liquid appear. These increase in size and become the vacuoles usually seen. Contributing vacuoles or channels such as occur in *Paramaecium* have never been noted. When they have reached sufficient size (10μ to 15μ in diameter in the ordinary individual), they change from their spherical shape and begin to bulge, each on the side toward the other. These bulges elongate until they meet at a midpoint. At this midpoint a new vacuole arises and into it, through the channels thus formed, the two vacuoles discharge their contents. This large middle vacuole almost immediately discharges to the exterior through the pellicle, and at the same time the other two vacuoles re-form. However, the discharge of the middle vacuole may be delayed until the other two are well formed and then the individual has three vacuoles present. This and other variations are not uncommon and should be taken into account in the use of the number of vacuoles as a basis for classification. Leuckart (1861) described a third contractile vacuole though he did not give its relative position to the other two and he did not describe the process of contraction. He reported that he had observed the vacuoles "drop-like" through the cytoplasm and wandering from place to place. Solojew (1901) described the two vacuoles and observed a canal connecting the two, but he did not explain its function in discharge of the contents.

Endoplasmic Structures

Endoplasm.—Within the ectoplasm the body is composed of the endoplasm (*end.*, pl. 27, figs. 7 and 8; pl. 28, figs. 9 to 12) and the inclusions therein. The endoplasm is less dense than the ectoplasm and more coarsely granular. It is quite fluid, having a fairly definite circulation in the active organism. From the inner end of the oesophagus the direction of flow is posteriorly along the ventral surface dorsalward just before reaching the posterior end, thence anteriorly along the dorsal surface. Just as it reaches the ectoplasm of the apical cone, it is deflected posteriorly down through the central portion of the body dorsal to the rootlets of the adoral cilia. This course can be followed readily by observing the circulation of the food vacuoles.

The *food vacuoles* are customarily globular and may contain starch granules, bacteria, or indigestible particles, of the food of the host. The ingestion of bacteria is probably abnormal since it seldom occurs except when the number of bacteria in the medium has increased greatly during incubation. The starch granules may occur in enormous numbers especially when the host has recently been fed on grain. Red blood cells have not been noted in any of the balantidia observed during the work.

That *Balantidium* may be cannibalistic is the only possible interpretation of several findings. In these instances small individuals were lying within the endoplasm of extremely large individuals ($100 \times 125\mu$) and were in a state of disintegration. The larger individuals were *Balantidium coli* in every case and the smaller may have been *Balantidium suis*, for both species were present in the material; but disintegration of the latter had progressed so far that specific identification was uncertain. The possibility of interpreting this phenomena as sporulation, which was described by Walker (1909), is precluded by the disintegration of the included organism and the fact that the normal vegetative phase of the nuclei of the large individual is unmodified. Further evidence is to be found in the fact that never more than a single individual has been found inside another, while sporulation would produce several.

Macronucleus.—As is generally the case in ciliates, balantidia are binucleate, having a large macronucleus and a small micronucleus. The macronuclei of *Balantidium coli* and *Balantidium suis* are slightly

different with respect to size and proportions as noted above (page 259), but structurally they are so closely alike that one description will suffice for both. The macronucleus (*mac.*, fig. 1, pl. 28, figs. 13 and 14) always lies in the endoplasm but otherwise is not constant in location. Immediately surrounding it is an area in which the endoplasm is less granular and less dense in appearance, due perhaps as Yocom (1918) has suggested of *Euplotes patella* to more rapid oxidation in this region. It is elongate and may be straight and rodlike or it may be sharply bent into a horseshoe shape. In any case its diameter increases toward either end, giving it something of a dumbbell shape. This constriction in the central region and enlargement at each end is more marked in *Balantidium coli* than in *Balantidium suis*. The nucleus is delimited by a definite nuclear membrane (*nuc. m.*, fig. 1) which is especially apparent in material in which the macronucleus has shrunk due to faulty technique. Within this membrane are packed rather densely the masses of chromatin. The chromatin never occurs in equal sized regular granules but rather in unequal very irregular masses, sometimes of considerable size (1 to 2μ in greatest dimension). Often there is a sort of vacuolated area, usually near one end of the macronucleus, which is free from chromatin. The significance of this vacuolated area I could not determine. It does not seem to be due to degeneration and is not related to any phase of reproduction. It is in no way comparable to the "reconstruction band" described by Griffin (1910) and by Yocom (1918) in *Euplotes worcesteri* and *Euplotes patella*, respectively. The chromatin stains black with haematoxylin so that the macronucleus is the most conspicuous structure in a stained individual. When Mallory's connective tissue stain is used, the macronucleus takes on an orange hue.

Micronucleus.—The micronuclei (*mic.*, fig. 1) of both species of *Balantidium* parasitic in pigs are exceedingly small, not exceeding 5μ in diameter. In the resting or vegetative phase the micronucleus is subspherical and its flattened side lies close against the nuclear membrane of the macronucleus. It may even lie in a depression in the macronucleus in which position it is scarcely distinguishable from the granules of the latter. It is surrounded by a nuclear membrane readily recognized when the micronucleus is undergoing mitosis. In a few instances the chromatin has appeared indistinctly granular but it is customarily so closely packed that it looks like a single solid mass.

Neuromotor Apparatus

The term neuromotor apparatus denotes an integrated system of fibers, with a coördinating center, which is present in some Protozoa, and which is credited with the power of conductivity of nervous impulses, and hence functions in the coördination of the motor organelles of the cell. The term was first used for ciliates by Sharp (1914) in his account of the structure of *Diplodinium ecaudatum*. Since then it has been employed by Kofoid and Christiansen (1915), Kofoid (1916) for flagellates, and by Yocom (1918) and by Taylor (1920) in their studies of the morphology and behavior of *Euplotes patella*.

The neuromotor apparatus of *Balantidium coli* can scarcely be considered apart from the motor organelles of the cell. To insure clarity in the description and discussion which follows, the motor organelles, previously described in some detail, will be briefly reviewed. With the exception of the oral plug which surrounds the cytostome the organism is thickly beset with cilia. Of these, the adoral cilia are largest. They are distributed about the margin of the peristome in a row which ends in the membranellar region in the ventral wall of the oesophagus. The rootlets of these cilia are exceedingly long, reaching well into the posterior third of the cell, where they end without any connection or attachment. They have two enlargements, the first being the basal granule which lies just beneath the pellicle, and the second being located at the junction of ectoplasm and endoplasm. The remainder of the cilia are arranged in longitudinal spiral rows. The most anterior cilia of these rows, i.e., those nearest the adoral cilia, are nearly as sturdy as the adoral cilia themselves. But, progressing posteriorly in the rows, the cilia become continuously smaller until they reach their minimum size at the base of the apical cone of ectoplasm. Likewise the ciliary rootlets become shorter, and the distance between the basal granules and the secondary enlargements of the rootlets becomes less and less as one approaches the base of the apical cone. This is shown in figure I. The remainder of the way posteriorly the cilia are of uniform size, and the secondary enlargement of the basal apparatus of each cilium is the termination of the ciliary rootlet and lies in the granular band of ectoplasm near its plane of junction with the endoplasm.

In *Balantidium coli* five distinct parts constitute the complete neuromotor apparatus, namely (1) a motorium or coördinating center, embedded in the ectoplasm close by the oesophagus, and from it fibers pass out to the oral plug and the motor organelles; (2) a circum-

oesophageal fiber, beginning and ending in the motorium and giving off branches as it passes through the oral plug; (3) a fiber connecting the adoral cilia and the cytostomal membranelles with the motorium; (4) the adoral ciliary rootlets passing posteriorly from the basal granules of the adoral cilia, each bearing an enlargement where it passes from ectoplasm into endoplasm, and finally ending without attachment in the endoplasm of the cell; (5) the radial fibers taking origin from the enlargements of the adoral ciliary rootlets and passing radially to the ectoplasmic layer where they turn posteriorly into the granular band.

The *motorium* (*mot.*, figs. I and J) when viewed in ventral aspect has somewhat the appearance of a reversed letter J. It lies within the ectoplasm of the apical cone, close to the ventral and right walls of the oesophagus. The part of the motorium corresponding to the vertical shaft of the J lies along the right wall of the oesophagus, and its anterior terminus is situated just inside and slightly dorsal from the point of origin of the right lip of the cytostome. The curved end of the J is posterior and passes ventrally and anteriorly around the oesophagus and ends close to the inner termination of the rows of adoral cilia. In the middle region a sharp slit-like constriction is often very conspicuous. It is with the part of the motorium anterior to this constriction, that the circumoesophageal fiber and the adoral ciliary fiber have their origin. The portion posterior to this constriction is quite variable both in size and in stainability. This variation is very suggestive of the behavior of the parabasal body of certain flagellates as described by Kofoid and McCulloch (1916), Swezy (1916), Kofoid and Swezy (1919). These authors have shown that the parabasal body is not kinetic in function but instead is a reserve or reservoir of material easily transformed into energy, which reservoir fluctuates according to the physiological condition of the animal. No direct evidence can here be advanced establishing such a function for this posterior portion of the motorium, but the fact of its fluctuation in volume and its variation in chemical nature (as shown by stains) would lead one to suspect that such an interpretation is correct.

The *circumoesophageal fiber* (*cir. oes. f.*, figs. I and J) takes origin from the anterior extremity of the motorium and passes into the oral plug. Its course is very close to the inner, i.e., oesophageal, surface of the plug. It encircles the oesophagus completely but in the membranellar area it becomes very hard to follow. Certain sections (pl. 27, fig. 3), however, seem to show that it unites again with the motorium. It is not a smooth fiber but bears irregular enlargements from which

fibers pass both posteriorly and anteriorly into the ectoplasmic mass of the oral plug. These branches make no observable connections, but seem to fade out in the ectoplasm. Morphological evidence would indicate that this portion of the neuromotor apparatus is concerned solely with the activities of the very mobile oral plug. The *adoral ciliary fiber* (*ad. cil. f.*, figs. I and J) also arises from the motorium. This fiber attaches to the motorium just anterior to the constriction. It passes directly to the basal granule of the first cilium of the adoral row from which it passes on and makes connection with each of the basal granules of the entire row. It turns inward following the row of adoral cilia along the dorsal or left-hand margin of the membranelle area. Here its course is exceedingly hard to determine, but like the circumoesophageal fiber, some sections seem to show its connection with the posterior end of the motorium.

The remainder of the neuromotor system is not directly connected with the motorium. The *adoral ciliary rootlets* (*ad. cil. r.*, fig. I) pass inward from the basal granules of the adoral cilia through the ectoplasm of the apical cone into the endoplasm and well into the posterior third of the organism where they end, not abruptly but by fading out. There is absolutely no indication of any attachment of their inner ends. Just posterior to the middle of the cell there is usually a very distinct crossing of the ciliary rootlets from the opposite sides of the peristome. Each of these adoral ciliary rootlets bears a decided enlargement at the point where it passes from the ectoplasm into the endoplasm. The aggregation of these enlargements gives the appearance in cross-sections through this region (pl. 27, fig. 6) of a zone of large, deeply staining granules about the oesophagus. Each of these enlargements gives rise to a fiber which passes radially outward in a transverse plane. These have been termed the *radial fibers* (*rad. f.*, fig. J; pl. 27, fig. 6). Each of these radial fibers as it passes peripherally connects with small enlargements of the rootlets of the cilia of the apical cone. The rootlets of the cilia of the apical cone are like those of the adoral cilia except that they shorten progressively as they near the periphery, at which point they scarcely extend into the endoplasm at all, becoming identical with those of body cilia. Since the radial fibers and the enlargements of the ciliary rootlets lie in the plane of contact between the ectoplasm and the endoplasm they very clearly mark the limit of the two. At the periphery the radial fibers turn posteriorly and become lost in the granular band. This last fact is very suggestive, since the terminal enlargements of the rootlets of the body cilia lie in these granular bands.

It has not been possible to demonstrate a posterior continuation of the radial fibers within the granular bands. If they do not continue posteriorly, making connections with the enlargements of the rootlets of the body cilia, then the logical alternative seems to be to attribute the function of conductivity to the granular band of ectoplasm itself. There must be some means of conduction of stimuli, for the body cilia are concerted in their action, though not to so high a degree as in the case of the cilia of the apical cone. Protoplasm is generally conceded to have the power of conducting stimuli. So here in *Balantidium coli* there seems to be a transition from conduction by the undifferentiated protoplasm which serves as a matrix for the ciliary apparatus, to a condition in which there is a differentiation of the protoplasm into fibers or strands to serve the purpose. Moreover, the increasing degree of differentiation is directly correlated with the increasingly high degree of coördination of the motor organelles.

It will be seen from the figures and the above description that *Balantidium coli* is equipped with a notably integrated system of fibers bearing such morphological relations as would make the assigning to them of the function of coördination perfectly logical. The motorium is connected directly only with the oral plug and the adoral cilia. But through ciliary rootlets and the radial fibers all of the cilia of the apical cone are in connection with the adoral cilia. And if the function of conductivity may be attributed to the granular bands of the ectoplasm, into which the radial fibers turn and become lost to view, and in which the enlargements of the ciliary rootlets of the body cilia lie, then all of the motor organelles of the organism are reached by the neuromotor apparatus, and by it the coördination of these organelles may be explained.

Attributing to the various fibers described above the quality of conductivity and to the whole neuromotor apparatus the function of coördination of all of the locomotor organs in swimming and feeding, microdissection experiments carried on by Taylor (1920), we may consider the functions of the various parts from that point of view. The main rôle of the motorium is to act as a coördination center. The circumoesophageal fiber would serve to correlate the movements of the oral plug with those of the adoral cilia in feeding and in avoiding-reactions. The ciliary rootlets and radial fibers make possible the coördination of all of the locomotor organs in swimming and feeding, and especially the cilia of the apical cone which are employed in the boring movement of the organism.

DISCUSSION

Up to the present time systems of intraeytoplasmic fibers and aeeessory neuromotor masses comparable to that found in *Balantidium coli* have been fully deseribed in several flagellates and a few ciliates. The very primitive type oecurring in *Naegleria gruberi* (Schardinger) has been described by Wilson (1916). Kofoid (1916) and Swezy (1916) have made a critical comparative study of the motor systems of those flagellates, in which they have been most carefully studied. That there is a striking similarity in the neuromotor systems of flagellates and ciliates is clearly pointed out by Yocom (1918).

Among the ciliates, intraeytoplasmie fibers have been known for some time and there have been several descriptions of them and several conflieting views as to their function (Engelmann, 1880; Bütschli, 1889; Schuberg, 1891; Maier, 1903; Prowazek, 1903; Griffin, 1910; Braune, 1913). Sharp (1914), however, was the first to deseribe fully a eompletely integrated fibrillar system with a central neuromotor mass, to which he applied the term neuromotor apparatus.

Of the neuromotor apparatus of ciliates that of *Balantidium coli* is the third to be quite fully worked out. In 1914 Sharp described the neuromotor apparatus of *Diplodinium ecaudatum* (Fiorentini). This apparatus consists of six parts. The central motor mass, or motorium, lies in the area of thickened ectoplasm at the anterior end of the animal between the dorsal and adoral membranelle zones. A fiber eonnects the motorium with the basal granules of the dorsal membranelles, a braneh from which runs along the base of the inner dorsal lip. Another fiber eonneets the motorium with the basal granules of the adoral membranelles. A set of opereular fibers leave the motorium and pass along underneath the operculum. Lastly, the motorium has a definite eonnection by means of a fiber with what Sharp called the circumoesophageal ring. There is also a set of fibers in the wall of the oesophagus, which he termed the oesophageal fibers, and which he believed took their origin from the circumoesophageal ring. All of these structures as well as the micronucleus Sharp found had an affinity for the acid fuchsin when Mallory's eonnective tissue stain was used.

In *Euplotes patella* (O. F. Müller) the neuromotor apparatus, as described by Yocom (1918), is made up of five distinct parts. The

motorium is a somewhat bilobed mass and lies in the ectoplasm at the anterior end of the organism close to the right anterior corner of the triangular cytostome. From the left end of the motorium five main longitudinal fibers pass posteriorly, diverging slightly, and each joins with one of the five anal cirri. The exact relation of these fibers to the basal plates of anal cirri has been very carefully determined by Taylor (1920). Leaving the right end of the motorium a fiber passes to the membranelles of the adoral zone. Directly connected with this fiber is the "sensory structure" of the anterior lip. Lastly, there are dissociated fibers in connection with the frontal, ventral, and marginal cirri. In *Euplotes*, as in *Diplodinium*, all parts of the neuromotor apparatus as well as the micronucleus stain brilliant red with acid fuchsin.

For facilitating comparison, it might be well to summarize briefly the neuromotor apparatus of *Balantidium coli*, as described above. It consists in this organism of five distinct divisions. The motorium is a J-shaped mass situated in the thickened ectoplasm of the anterior end of the animal close to the cytostome and oesophagus. From it arises the circumoesophageal fiber, which gives off branches to the oral plug. A second fiber takes its origin from the motorium and connects with the basal granules of the adoral cilia. The adoral ciliary rootlets pass inward from the basal granules of the adoral cilia, and may extend well into the posterior third of the cell. The radial fibers take their origin from enlargements of the adoral ciliary rootlets, at the point where they enter the endoplasm. They pass outward radially, making connection with the ciliary rootlets of the cilia of the apical cone, and at the periphery turn posteriorly and cannot be traced farther in the granular bands of the ectoplasm. As in the previous forms just summarized, the neuromotor apparatus as well as the micronucleus of *Balantidium coli* is selective for acid fuchsin.

Of the three examples of neuromotor systems so far fully worked out and described, each represents a different order of the class Ciliata: *Diplodinium* being of the order Oligotricha; *Euplotes* being of the order Hypotricha; and *Balantidium* being of the order Heterotricha. Yet in spite of the diversity of forms there is a remarkable similarity in the neuromotor system. The presence of a motorium is common to all three. In each of the three organisms it is located at the anterior end of the animal and lies wholly within the ectoplasm near the cytostome. By means of fibers it is connected with a part or all of the motor organelles of the animal. Another feature of the

fibrillar portion of the apparatus common to all three forms is the strand connecting the motorium with all of the adoral cilia or membranelles, as the case may be. Finally, the system, in all cases, shows an affinity for acid fuchsin.

The circumoesophageal ring present in *Diplodinium* is represented in *Balantidium* by the circumoesophageal fiber running through the oral plug. The oral plug forms the wall of the greater part of the oesophagus, and the fiber lies very close to the oesophageal surface. From this fiber there are given off fibers which pass both anteriorly and posteriorly in the mass of the oral plug. These fibers are strikingly similar in location to the oesophageal fibers, described by Sharp (1914) in the wall of the oesophagus of *Diplodinium* and believed by him to arise from the circumoesophageal ring. Sharp points out that these fibers approach very close to the micronucleus though there was no demonstrable connection with it. In *Balantidium* this possibility is precluded by the shortness of the oesophagus which in no case extends inward to a point anywhere near the micronucleus. At times when the organism is viewed from the proper angle, the ciliary rootlets of the adoral cilia may have the appearance of ending in the proximity of the micronucleus and suggesting a connection with it. No such connection exists, however, as may be readily demonstrated in large numbers of whole mounts and still better in sections where these rootlets may be traced far posterior to the micronucleus, passing it some considerable distance away. In the vegetative phase, it is certain that no structural connection exists between any part of the neuromotor apparatus and the micronucleus.

Of the three neuromotor systems of ciliates here considered, that of *Balantidium* is clearly the least centralized, though none the less a unified structure. In *Diplodinium* all motor organelles connect directly with the motorium, and in *Euplotes* the same is true of all except the marginal cirri which have no connection whatever, whereas in *Balantidium* only the adoral cilia have direct connection with the motorium while the cilia of the apical cone (and those of the body, too, if they have any connection whatever) are connected with it only indirectly through the radial fibers and the rootlets of the adoral cilia. This lesser degree of centralization of the neuromotor apparatus may perhaps be explained by the lesser degree of specialization of locomotor organelles. Whereas in *Diplodinium* the body is devoid of cilia and the dorsal and adoral zones of membranelles are the sole motor organelles, and in *Euplotes* the locomotor organelles are restricted to the cytostomal membranelles and a few cirri on the ventral surface, in

the case of *Balantidium* the entire organism, with the exception of the oral plug, is covered with cilia. So the slightly lesser degree of specialization in the neuromotor apparatus would be the logical expectation if modification of intracytoplasmic structures is correlated with modification of external structures with which they have a direct connection or of which they form an integral part.

From the point of view of efficiency, also, the arrangement in *Balantidium* is readily explainable. The locomotor activities of the organism may be separated into three main sorts, swimming, feeding, and boring, this last very likely being used in penetration of the intestinal wall of the host. In swimming the coördination of the entire locomotor apparatus is necessary. In feeding, and in boring, particularly, the coördination of the adoral cilia with those of the apical cone is extremely essential. Such coördination would be most effectively brought about by a direct connection of the parts concerned, and this direct connection is accomplished by the uniting of all of the rootlets of the cilia of these two regions by means of the radial fibers, without the interpolation of the motorium.

Throughout the above discussion the assumption of a neural function for the neuromotor apparatus, i.e., the power of conductivity of stimuli resulting in coördination of parts, has been based on two general types of evidence, morphological and experimental. The chemical evidence, that is, the affinity for acid fuchsin, as presented by Sharp (1914) for *Diplodinium* and by Yocom (1918) for *Euplotes*, seems slightly less convincing in the case of *Balantidium*. In the last named organism not only does the micronucleus, which has no connection with the neuromotor apparatus, show an affinity for acid fuchsin but so also do certain cytoplasmic inclusions, which if not food particles are at least undoubtedly concerned in some way with metabolism and have no morphological relation to the neuromotor apparatus. Yocom (1918) states that there is more of the orange G in the micronucleus giving it a different shade from the parts of the neuromotor apparatus in the case of *Euplotes;* but in *Balantidium* I have been unable to detect any such differentiation.

Morphological evidence for attributing neural function to the neuromotor apparatus has been clearly presented by Yocom (1918) in his discussion of the apparatus in *Euplotes*. The evidence found in *Balantidium* is not strikingly different. There is in the latter organism the same intricate relationship between the neuromotor apparatus and the motor organelles. The most active cilia, i.e., the adoral cilia, are directly connected by the adoral ciliary fiber with

each other and with the motorium. The cilia of the apical cone are connected with the adoral cilia by the radial fibers and so indirectly with the motorium. In this case, however, coördination with the adoral cilia is most essential and this corresponds with their intimate connection. These morphological interrelations all point to a neural function for the neuromotor apparatus.

Very convincing experimental evidence of the neural function is to be found in the results of Taylor's (1920) microdissection experiments on *Euplotes patella.* In *Euplotes* there is a fiber connecting the adoral membranelles with the motorium, and also a fiber connecting each of the five anal cirri with it. In a series of experiments Taylor severed various ones of these fibers and observed very carefully the effect on the movements of the animal. He then compared these movements with the normal movements which he had previously carefully analyzed and classified. Severing of these fibers resulted in lack of coördination of the parts thus disconnected and resulted in abnormal movements. Incision made in other parts of the cell, but which did not sever neuromotor fibers did not so result. In the words of the author, "It is apparent, then, that the destruction of the motorium or the severing of some or all of its attached fibers is alone accountable for modification in the perfect and efficient coördination between the series of membranelles and the anal cirri. We may, therefore, regard these normal morphological relationships as conditioning the animal's usual behavior both in creeping and in swimming."

The general occurrence among Protozoa of protoplasmic modification to form organelles for locomotion, feeding, digestion, excretion, and protection has been known almost as long as have the Protozoa themselves. The functions of such organelles have not been difficult to determine. One might equally well expect to find modifications correlated with the conduction of stimuli, but the establishing of neural function is not so easy. This difficulty in conjunction with the traditional idea of the simplicity of the Protozoa has resulted in conservatism in crediting any intracellular structures with the function of conductivity. The recent detailed morphological studies on Protozoa and the experimental work of Taylor (1920), however, leave little doubt regarding the matter. This account of the neuromotor apparatus of *Balantidium coli* and *Balantidium suis* presents additional evidence of the likelihood of a quite general occurrence, in the Protozoa, of intracytoplasmic specialization resulting in a more or less integrated system for purposes of coördination.

SUMMARY

1. Pigs are very generally infected with balantidia as shown by the findings in previous investigations, many of which were carried on in foreign countries, and by the finding of 68 per cent infection among the two hundred pigs examined during the present investigation.

2. There are two species of the genus *Balantidium* that are parasitic in the intestinal tract of pigs, namely, *Balantidium coli* and *Balantidium suis* (sp. nov.).

3. *Balantidium coli* is the species first described by Malmsten (1857) from man, and later by Leuckart (1861) as a parasite in pigs.

4. *Balantidium suis* (sp. nov.) has not hitherto been distinguished from *Balantidium coli*. The former differs from the latter in being more elongate and being broadest anterior instead of posterior to the equatorial plane; in having a more slenderly proportioned macronucleus; and in having the mouth displaced ventrally, instead of being almost terminal, which causes the plane of demarcation between ectoplasm and endoplasm in this region to slant posteriorly toward the ventral surface instead of being perpendicular to the longitudinal axis, as is the case in *Balantidium coli*.

5. So far as recorded facts will justify conclusions, it seems unlikely that *Balantidium suis* occurs as a parasite of man, but instead that *Balantidium coli* is the cause of balantidiasis. Whether or not such is the case, it is very desirable that the occurrence of the two species in pigs be taken into account in future work on experimental infection, for to a failure to distinguish between the two species may be due the seemingly conflicting results of previous experiments.

6. The cilia of the two species are homologous. Variation occurs in size and relative position of parts only. The basal apparatus is essentially diplosomic, consisting of a basal granule connected by a ciliary rootlet to a secondary enlargement. The former is situated just beneath the pellicle; the latter lies in the plane of demarcation between ectoplasm and endoplasm. The adoral cilia are largest, the basal granule and secondary enlargement are farthest removed from one another, as the ectoplasmic thickening is greatest in this region, and the ciliary rootlet may extend far into the endoplasm. The cilia of the apical cone intergrade between the adoral cilia and the body cilia. As one

progresses posteriorly, they become smaller in size, the secondary enlargement approaches the basal granule as the ectoplasmic layer becomes thinner, and the extension of the rootlet into the endoplasm becomes shorter. The body cilia are smallest and their rootlets terminate in the secondary enlargement. The cilia of the anterior end are highly concerted in action. They beat in such a way as to give the animal a remarkable boring motion which probably serves in the penetration of the mucosa of the intestine.

7. Both species possess a neuromotor system. This is a highly developed and integrated system consisting of five correlated parts. The motorium, lying within the ectoplasm near the cytostome, gives rise to a circumoesophageal fiber. In addition, there is a heavier fiber which connects it with the basal granules of the adoral cilia. The rootlets of the adoral cilia extend far into the endoplasm, usually well into the posterior one-third of the cell. Where they pass from ectoplasm into endoplasm each adoral ciliary rootlet bears an enlargement and from this arises a radial fiber which passes to the periphery, turns posteriorly, and disappears in the granular band of ectoplasm. In passing to the periphery these radial fibers connect with enlargements of the rootlets of the cilia of the apical cone.

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LITERATURE CITED

Bel, G. S., and Couret, M.

1910. *Balantidium coli* infection in man. Jour. Infect. Dis., **7**, 609–624, 4 pls.

Bezzenberger, E.

1904. Ueber Infusorien aus asiatischen Annren. Arch. f. Prot., **3**, 138–174, pl. 1, 23 figs. in text.

Boeck, W. C.

1917*a*. Mitosis in *Giardia microti*. Univ. Calif. Publ. Zool., **18**, 1–26, pl. 1.

1917*b*. A rapid method for the detection of protozoan cysts in mammalian faeces. *Ibid.*, **18**, 145–149.

Braune, R.

1913. Untersuchungen über die im Wiederkänermagen vorkommenden Protozoan. Arch. f. Prot., **32**, 5–63, pls. 3–6.

Brumpt, E.

1909. Demonstration du rôle pathogène du *Balantidium coli*. C.-R. Soc. Biol., Paris, **67**, 103.

Bütschli, O.

1889. "Ciliata" *in* "Protozoa" (1887–1889) *in* Bronn, Klass. und Ordn. des Thierreichs, **1**, (3), 1228–1841, pls. 56–86.

Chagas, C.

1911. Über die zyklischen Variationen des Caryosomes bei zwei Arten parasitischer Ciliaten. Mem. Inst. Oswaldo Cruz, **3**, 136–144, 2 pls.

Claparède, E., and Lachmann, J.

1858. Études sur les infusoires et les rhizopodes. (Genève, Kossmann), **1**, 247–248, pl. 13.

Delage, Y., and Hérouard, E.

1896. Traité de zoologie concrete. (Paris, Schleichter Frères), **1**, 582, 870 figs. in text. Ref. footnote, 404, fig. 692.

Dobell, C. C.

1909. Researches on the intestinal Protozoa of frogs and toads. Quart. Jour. Micr. Sci., **53**, 201–277, 4 pls.

Doflein, F.

1911. Lehrbuch der Protozoenkunde. (Jena, Fischer), ed. 3, xii + 1043, 951 figs. in text.

Ehrenberg, C. G.

1838. Die Infusionsthierchen als volkommene Organismen. (Leipzig, Voss), xviii + 547; Atlas, pls. 1–64.

Englemann, T. W.

1880. Zur Anatomie und Physiologie der Flimmerzellen. Pflüger's Arch. ges. Physiol., **23**, 505–535, pl. 5.

Griffin, L. E.

1910. *Euplotes worcesteri* sp. nov., 1, Structure. Philippine Jour. Sci., **5**, 291–312, pls. 1–3, 13 figs. in text.

Jennings, H. S.

1908. Heredity, variation and evolution in Protozoa. Proc. Am. Phil. Soc., **47**, 393–546.

Johnson, H. P.
1893. A contribution to the morphology and biology of the stentors. Jour. Morph., **8**, 467–562, pls. 23–24.

Kofoid, C. A., and Christiansen, E. B.
1915. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–54, pls. 5–8, 1 fig. in text.

Kofoid, C. A., and McCulloch, I.
1916. On *Trypanosoma triatomae*, a new flagellate from a hemipteran bug from the nests of the wood rat *Neotoma fuscipes*. *Ibid.*, **16**, 113–126, pls. 14–15.

Kofoid, C. A., and Swezy, O.
1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Am. Acad. Arts and Sci., **51**, 289–378, pls. 1–8, 7 figs. in text.
1919. On *Trichonympha campanula* nov. sp. Univ. Calif. Publ. Zool., **20**, 41–98, pls. 5–12, 4 figs. in text.

Leeuwenhoek, A. von.
1708. "Animalcula e stercore ranarum." Opera omnia, **2**, 49–64.

Leuckart, R.
1861. *Paramoecium coli*. Wiegemann's Archiv, **1**, 80, pl. 5.

Long, J. A.
1912. Studies on early stages of development in rats and mice. Univ. Calif. Publ. Zool., **9**, 105–136, pls. 13–17, 11 figs. in text.

Maier, H. N.
1903. Ueber den feineren Bau der Wimperapparate der Infusorien. Arch. f. Prot., **2**, 73–179, pls. 3–4.

Malmsten, P. H.
1857. Infusorien als Intestinaltiere beim Menschen. Virchow Arch. f. pathol. Anat., **12**, 302–309, pl. 10.

Maupas, E.
1883. Contribution à l'étude morphologique et anatomique des infusoires cilies. Arch. Zool. Exp. et Gen., (2), **1**, 427–664, pls. 19–24.

Metcalf, M. M.
1908. *Opalina*—anatomy and reproduction. Arch. f. Prot., **13**, 195–375, pls. 14–28.

Minchin, E. A.
1912. An introduction to the study of the Protozoa. (London, Arnold), xi + 520, 194 figs. in text.

Neresheimer, E. R.
1903. Die Höhe histologischer Differenzierung bei heterotrichen Ciliaten. Arch. f. Prot., **2**, 305–324, pl. 7, 1 fig. in text.

von Prowazek, S.
1903. Flagellatenstudien; Anhang; fibrilläre Strukturen der Vorticellen. *Ibid.*, **2**, 195–212, 2 pls.
1913. Zur Kenntnis der Balantidiosis. Arch. f. Schiffs-u. Tropenhyg., **17**, 369–390, 2 pls., 9 figs. in text.

Pütter, A.
1903. Die Flimmerbewegung. Ergebn. d. Physiol., **2**, 1–102, 15 figs. in text.

Saguchi, S.
1917. Studies on ciliated cells. Jour. Morph., **29**, 217–268, pls. 1–4, 1 fig. in text.

Schaudinn, F., and Jakoby, M.
1899. Ueber zwei neue Infusorien im Darm des Menschen. Centralbl. f. Bakt., Abt. I, **25**, 487–494, 4 figs. in text.

Schuberg, A.
1886. Ueber der Bau der *Bursaria truncatella* mit besonderer Berücksichtigung der protoplasmatischen Structuren. Morph. Jahrb., **12**, 333–365, pls. 19–20.
1891. Ueber einige Organizationsverhältnisse der Infusorien des Wiederkauermagens. Sitz.-ber. d. phys.-med. Ges. Wurzburg, **1891**, 122–137.

Schweier, A.
1900. Parasitische Wimperinfusorien (Endoparasiten). Arb. der St. Petersburger naturf. Gesellsch., **29**, 1–135, pls. 1–2.

Sharp, R. G.
1914. *Diplodinium ecaudatum*, with an account of its neuromotor apparatus. Univ. Calif. Publ. Zool., **13**, 43–122, pls. 3–7, 4 figs. in text.

Solojew, N. S.
1901. Das *Balantidium coli* als Erreger chronischer Durchfalle. Centralbl. f. Bakt., **29**, 321–830; 849–860, 8 figs. in text.

Stein, F.
1867. Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. (2) Naturgeschichte der heterotrichen Infusorien (Leipzig, Englemann), viii + 355, 16 pls.

Strong, R. P.
1904. The clinical and pathological significance of *Balantidium coli*. Bureau Gov't. Labs. (Manila, P. I.), Bull. **26**, 77, 10 pls.

Swezy, O.
1916. The kinetonucleus of flagellates and the binuclear theory of Hartmann. Univ. Calif. Publ. Zool., **16**, 185–240, 58 figs. in text.

Taylor, C. V.
1920. Demonstration of the function of the neuromotor apparatus in *Euplotes* by the method of microdissection. *Ibid.*, **19**, 403–470, pls. 29–33, 2 figs. in text.

Thon, K.
1904. Ueber den feineren Bau von *Didinium nasutum* O. F. M. Arch. f. Prot., **5**, 281–321, pls. 12–13, 3 figs. in text.

Walker, E. L.
1909. Sporulation in the parasitic Ciliata. *Ibid.*, **17**, 297–306, 2 pls.
1913. Experimental balantidiasis. Philippine Jour. Sci., **8**, (B), 333–349, 7 pls.

Wilson, C. W.
1916. On the life history of a soil amoeba. Univ. Calif. Publ. Zool., **16**, 241–292, pls. 18–23.

Wising, P. J.
1871. Till kännedomen om *Balantidium coli* hos människan. Nordiskt Med. Ark., **3**, 1–30, 1 pl.

Yocom, H. B.
1918. The neuromotor apparatus of *Euplotes patella*. Univ. Calif. Publ. Zool., **18**, 337–396, pls. 14–16, 1 fig. in text.

EXPLANATION OF PLATES

PLATE 27

Camera lucida drawings of a series of transverse sections of *Balantidium coli* (Malmsten). The series begins at the anterior end and progresses posteriorly about one-third the length of the organism. × 1000.

Fig. 1. This section shows dorsal portion only of peristome. The adoral cilia are removed showing their basal granules clearly.

Fig. 2. A portion of the oral plug is shown with the circumoesophageal fiber and the connected oral plug fibers. The deeply-staining region between protoplasm of oral plug and the surrounding ectoplasm, so marked in this individual, is rarely found.

Fig. 3. The continuation of cilia over the ventral lip and down into the cytostome are to be noted particularly; the beginning of the motorium on the (reader's) left of the cytostome; and the connection of the adoral ciliary fiber with the motorium.

Fig. 4. In this figure the cilia of the cytostome give the appearance of membranelles; the circumoesophageal fiber makes connection with the motorium.

Fig. 5. This shows the beginnings of the enlargements of the adoral ciliary rootlets.

Fig. 6. Here the oesophagus is completely surrounded by the enlargements and radial fibers may be seen arising from some of them.

Fig. 7. The extreme posterior tip of the motorium appears within the circle of enlargements; the dorsal half of this section is through the endoplasm.

Fig. 8. This section is posterior to the adoral apparatus with the exception of the rootlets of the adoral cilia which in cross-section can not be distinguished from the granules of the endoplasm.

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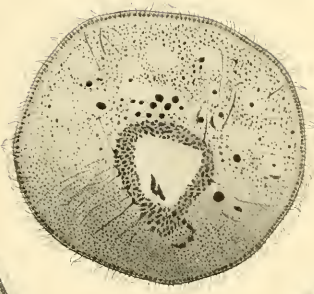
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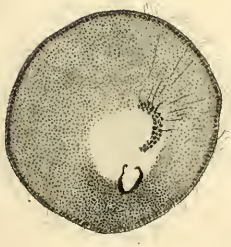
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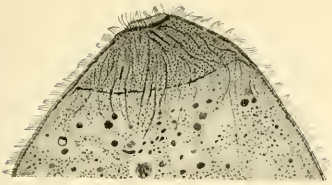
PLATE 28

Figs. 9–12. Series of longitudinal sections through the anterior end of *Balantidium coli* (Malmsten). × 1000. These sections are oblique, being more nearly frontal than sagittal, however. The section shown in figure 9 is tangential to the margin of the peristome some distance to the right of the most dorsal point of the latter (see fig. J in text). The remainder progress toward the left ventral lip. The oral plug fibers, the radial fibers, and the adoral ciliary rootlets are shown clearly in these sections.

Fig. 13. A sagittal section through *Balantidium suis* (sp. nov.). × 1000. The specific characters, viz., elongate body and macronucleus, and *oblique* plane delimiting apical cone of ectoplasm, are shown in this section. The cytopyge is distinct and open to the exterior. A paramylum body of considerable size is present in the endoplasm.

Fig. 14. An oblique longitudinal section through *Balantidium coli* (Malmsten) showing the protrusion of the oral plug. × 750.



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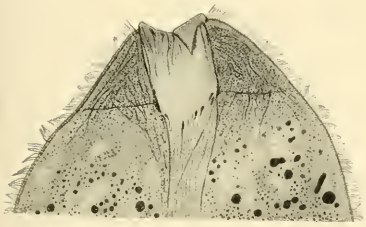

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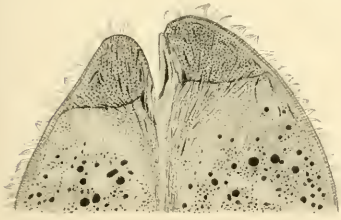

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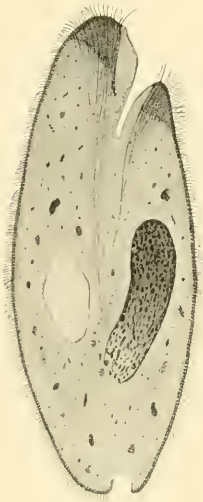

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BY
CHARLES ATWOOD KOFOID AND OLIVE SWEZY

ENDAMOEBA DYSENTERIAE IN THE LYMPH GLANDS OF MAN IN HODGKIN'S DISEASE

BY
CHARLES A. KOFOID, LUTHER M. BOYERS, M.D.,
AND OLIVE SWEZY

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MITOSIS IN ENDAMOEBA DYSENTERIAE IN THE BONE MARROW IN ARTHRITIS DEFORMANS

BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

(Contribution from the Zoological Laboratory, University of California, and from the Division of Parasitology, Bureau of Communicable Diseases, California State Board of Health)

The coincidence of the presence of *Endamoeba dysenteriae* in the human bowel and a diagnosis of *arthritis deformans* has been a matter of observation and record in our laboratory for several years. We have previously called attention to this relation to rheumatism of the joints (Kofoid, 1920). We later (1922) identified amoebae in the bone marrow in material submitted by Dr. Ely in Ely's (1920) second type of *arthritis deformans*, and our discovery was announced coincidently by Ely, Reed, and Wyekoff (1922) with ourselves. This note is a preliminary statement of the proof that the cells interpreted by us as amoebae are in reality parasitic rhizopods and not amoeboid human cells.

The proof rests upon the fact that mitosis in the Rhizopoda and in the Metazoa, including that in human cells, is of two distinct types, clearly distinguishable by well-known cytological criterions of such definiteness that there can be no possibility of any trained cytologist or protozoologist confusing them. If the amoeboid cells found by us in the bone marrow of the lesions in the excised head of the femur are really amoebae, they will exhibit, when they divide, the characteristic behavior and resulting morphology in fixed and stained material which occurs in the rhizopods. If they are only wandering amoeboid human cells, they will exhibit the human type of mitosis, or, if the tissue is degenerating, some modification of it. It is hardly probable or possible in the light of known degenerating human cells that degen-

eration should reproduce in each instance which we have detected of division in the supposed amoebae, the rhizopod type of mitosis.

Through the courtesy of Dr. C. L. Ely of Stanford University Medical School, unstained sections and undecalcified portions of the excised head of the femur of his case 187 of the second type (non-bacterial) of *arthritis deformans* have been placed in our hands for investigation. This excised bone had been fixed promptly in formalin

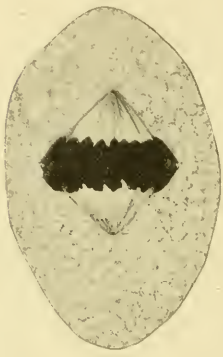


Fig. 1

Fig. 2

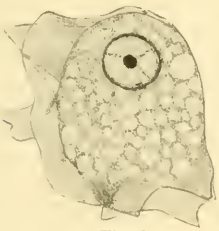
Fig. 1. Lateral view of a small epithelioid (?) cell in the metaphase of mitosis from a hypertrophied cervical gland of man in Hodgkin's disease. Note the absence of nuclear membrane, and the spindle fibers running from the centrosomes at the apices to the massed, deeply stained chromosomes in the equatorial plate. From a section stained in iron haematoxylin. × 2500.

Fig. 2. Lateral view of the anaphase of mitosis in a similar cell from the same source. × 2500. Note the elongated divided chromosomes retreating towards the poles of the spindle, and the lateral constriction, indicating approaching separation of the two daughter cells. Only part of the chromosomes (about 16) of the cell have been drawn, because of the superposition of the deeply stained rods which form the chromosomes. We are indebted to Dr. Doxey Wilson and Dr. Eastman of San José for the material from which these sections were made.

and was well preserved, though difficult to stain, and somewhat difficult to decolorize normally. The cells are splendidly preserved and the tissues came through decalcification with little if any disturbance.

Prolonged and assiduous search among the amoeboid cells which we interpreted as amoebae has brought to light several instances of stages of mitosis and several binucleate amoebae, indicating that asexual binary fission was in progress among the amoebae in the margins of the necrotic areas in the head of the femur.

We have also found dividing human cells in the hypertrophied cervical gland excised from a case of Hodgkin's disease. We have not found human cells of any kind dividing in the lesions of the bone. A comparison of the two types of division clearly indicates that the



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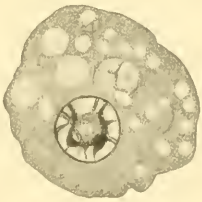

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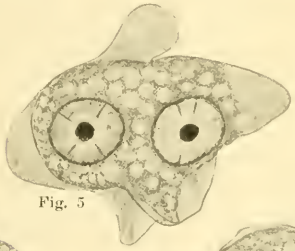

Fig. 5

Fig. 6

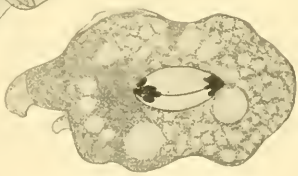

Fig. 7

Fig. 3. *Endamoeba dysenteriae* from lesions in bone marrow in the head of the femur in Ely's case 187 of the second, non-bacillary type of arthritis. Note spherical vesicular nucleus with peripheral chromatin, central karyosome, and spoke radiations.

Fig. 4. Same with nucleus in prophase with peripheral chromatin in strands (chromosomes?), some of them split.

Fig. 5. Binucleate amoeba resulting from mitosis. Halo around central karyosome.

Fig. 6. *Endamoeba dysenteriae* in early anaphase. Note small number of chromosomes, probably six, intact nuclear membrane and intradesmose joining centrosomes at poles.

Fig. 7. Late anaphase with distinct intradesmose. All figures × 2500 diameters, from sections of the excised head of the femur in Ely's case 187.

two types are quite distinct and that the type in the cells under suspicion is distinctly that of the rhizopod rather than that of the mammalian or human cell.

Two human cells from the lymph gland are shown in the metaphase and anaphase of mitosis in figures 1 and 2.

The human cell at mitosis is characterized by three fundamental structural features which are common to mammalian, if not to metazoan cells, at mitosis. These are, first, the entire disappearance of all traces of the nuclear membrane. This leaves the spindle of the dividing cell with its equatorial (fig. 1) or divided (fig. 2) group of chromosomes exposed free in the enveloping cytoplasm. In the comparable stages in the mitosis of amoeba (figs. 6 and 7), the whole spindle is contained within the persistent nuclear membrane. This membrane can be distinctly followed in focussing as a slightly stained wall surrounding the spindle. The spindle lies within it, its spindle fibers being few (fig. 6) in comparison with the many fibers of the human spindle (figs. 1 and 2) and lightly stained. The centrosomes are minute dots at the poles (fig. 6) within the nuclear membrane of the dividing nucleus of amoeba.

A second point of difference is the absence in the human spindle during the period of late prophase to the close of the anaphase of a deeply staining strand joining the centrosomes which we (1921) have called the intradesmose. The intradesmose is formed from the parental centrosome and as this divides and the resulting daughter centrosomes move to the poles of the spindle, a deeply staining substance similar, in iron haematoxylin, to that in the polar masses which constitute or contain the centrosomes, is spun out in a meridional thread often applied to the inner face of the nuclear membrane. If heavily decolorized, this is destained. If laterally located, it may be difficult to detect. In favorable material (fig. 7) it is clearly seen. This structure has been found by us in dividing nuclei in the intestinal cysts of *Endamoeba dysenteriae* (not yet published), in *E. coli* (Swezy, 1922, in press) and in *Councilmania lafleuri* (Kofoid and Swezy, 1921). It corresponds to the extranuclear paradesmose of the flagellates, and to the axial strand of linin fibers in the mitotic spindle of the dividing metazoan cell. In the dividing human cells, from the lymph glands of Hodgkin's disease, no structure such as the intradesmose can be found. In fact, none is known in cells of mammals, nor, in so far as we know, in any metazoan cells. In the Protozoa, it occurs in the Rhizopoda.

A third criterion exists in the number of chromosomes. The number of chromosomes in man appears to be either 24 or 48, or possibly both, the former being the diploid and the latter, the tetraploid condition. Guyer (1910, 1914) reported 22, but Montgomery (1912) and Wieman (1917) raised the number to 24. However, double this number have been reported by Evans (1918, 1921) and Painter (1921) who suggest 45 to 48. The finding of 47 chromosomes by Winiwarter (1917) in the testis suggests the possibility that there may be 47 in the male and 48 in the female in the tetraploid phase, and, by inference, in the diploid phase 23 and 24.

We have found (Kofoid and Swezy, 1921) 8 chromosomes in *Councilmania lafleuri*, and 6 (see also Swezy, 1922, in press) in *Endamoeba coli*, and probably 6 (not less than 5 nor more than 7) in *Endamoeba dysenteriae*. The number of chromosomes in the dividing cells which we interpret as dividing amoebae is not 24, much less 48. It is at the most a small number (fig. 6), probably 6. The number in the dividing human cells observed by us in lymph glands of Hodgkin's disease (figs. 1 and 2) is many more than 6. The chromosomes are so crowded in the equatorial plate (fig. 1) or so overlie each other, especially at the margins of the divided equatorial plate in lateral view, that the exact number can not be ascertained with certainty. We have not yet found a polar view in which the practicability of counting will be enhanced. The individual chromosomes are indicated by the projecting lobes representing their free ends. The number of these free ends on either side (fig. 2) or of elbows on the polar side of each group may be estimated in the most favorable region, namely, a sector of 90° on one surface. The number in our sections appears to be not less than 24, and not the tetraploid (?) 48. It is certainly not 6. In view of the general acceptance by cytologists of the view that the somatic chromosome number is both constant and characteristic, we are constrained to believe that this criterion is valid and critical to determine the non-human and the amoebic identity of these cells.

The chromosomes of the human cells are also larger and proportionately longer than those of the amoebae. They are consistently so in all the mitotic figures at the metaphase or near it of the amoebae which we have been able to detect, and we find no evidence in our material which connects these types of mitosis with any observed form of degenerating human cells. They appear to be normal parasitic amoeba in the normal phases of mitosis.

The presence of the amoebae in the lesions of Ely's second or non-bacterial type of *arthritis deformans* supplies the missing etiological factor in this disease. The term, amoebiasis of the bones, would therefore more accurately link the disease with its causative organism, in the event that an etiological relationship is confirmed by future work.

The question as to the exact species of amoeba found in the bone lesions is perhaps still an open one. It can not be *Councilmania lafleuri*, which has eight chromosomes and a granular karyosome. The only amoebae definitely known to be tissue invaders are *Endamoeba dysenteriae* and *E. gingivalis*. Mitosis and chromosome number in the latter are unknown. In our material and in published accounts of this species, the morphology of the nucleus differs in one minor particular, the absence of spoke-like radiations. In view of this fact, we tentatively incline to the view that the amoeba in the bone lesions is *E. dysenteriae* rather than *E. gingivalis*, on the assumption and perhaps rather widely accepted view that the two are distinct species. There is no satisfactory evidence that they are identical, though evidently rather more closely related in morphology to each other than is either to any of the other amoebae of man.

SUMMARY

Active amoeboid cells with the type of nucleus found in the cysts and active stages of *Endamoeba dysenteriae* in the bowel and in active amoebae in the lesions of amoebiasis are found in the lesions of the bones in *arthritis deformans* of Ely's second or non-bacterial type. These amoebae at mitosis have an intact persistent nuclear membrane, a meridional intradesmose, and a small number of chromosomes, probably six. This type of mitosis is that of the Rhizopoda, not of the Metazoa, Mammalia or man. It is in these details exactly similar to that found in *E. dysenteriae* of the bowel. Human cells at somatic mitosis have, excluding sex differences, 24 (diploid) or 48 (tetraploid?) chromosomes, no intradesmose, and the nuclear membrane disappears. The mitoses in the amoebae appear to be normal, and no intergrades with degenerating human cells have been found. The amoeba of the bone lesions appears to be *E. dysenteriae* rather than *E. gingivalis* of the gingival abscesses and tonsils. The morphological evidence supports the conclusion that *E. dysenteriae* is the

parasitic etiological factor in Ely's second type of *arthritis deformans*. This disease might therefore be designated as amoebiasis of the bones provided the etiological relationship of the parasite to the disease can be established.

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ENDAMOEBA DYSENTERIAE IN THE LYMPH GLANDS OF MAN IN HODGKIN'S DISEASE

BY

CHARLES A. KOFOID, LUTHER M. BOYERS, M.D., AND OLIVE SWEZY

(Contribution from the Zoological Laboratory, University of California, and the Division of Parasitology, Bureau of Communicable Diseases, California State Board of Health)

Hodgkin's disease has come to be known since the investigations of Reed (1902) and others as a clinical and pathological entity with a specific histological picture, not a simple hyperplasia, but with changes suggesting a chronic inflammatory process. The pathological agent, however, is unknown. It is the purpose of this preliminary note to make an announcement of the discovery of *Endamoeba dysenteriae* in the lesions of the lymph glands in this disease. In a previous communication (1922), we announced the coexistence of intestinal amoebiasis with the cysts of *Endamoeba dysenteriae* in the stools, and a diagnosis of Hogdkin's disease in the first two cases we examined, with a probability of the coexistence in a third (Lincoln's case). The mathematical probability of such a coincidence in the first three cases on the basis of an incidence of amoebiasis at large of 4 per cent is 1 in 15,625. On the basis of 1 per cent incidence, it is 1 in 1,000,000. Since then a fourth case has been examined with an added instance of coincidence, thus increasing the probability of an etiological connection between this parasite and this disease of hitherto unknown cause.

This last case was an inguinal gland apparently but recently attacked by the disease. Sections of this gland have been stained in iron haematoxylin and systematically searched for evidences of amoebic infection. In the territory of the gland most involved in the progress of the pathological modifications due to the disease we have found amoeboid cells with vesicular nuclei which we interpret as amoebae (figs. 1, 2, and 3).

As has been stated in the previous article, more evidence is required to establish a critical determination of these suspected cells as parasitic amoebae, since they may be only wandering amoeboid cells with an unusual type of nucleus for a human cell, a product of the pathogenic process.

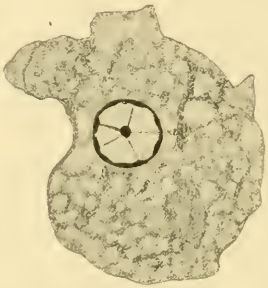

Fig. 1

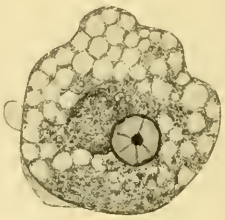

Fig. 2

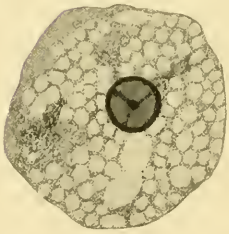

Fig. 3

Fig. 4

Figs. 1–3. *Endamoeba dysenteriae* in the active state with pseudopodia, spherical vesicular nucleus with peripheral chromatin, central karyosome, and spoke radiations. × 2500.

Fig. 4. An amoeba with nucleus in the telophase of cell division, with intact nuclear membrane, a few (probably not over six) chromosomes at either pole. The intradesmose is the black line connecting the two masses of chromosomes. It presumably connects the centrosomes at the apices of the nucleus. × 2500. All drawings from amoebae in sections of inguinal gland stained in iron haematoxylin. We are indebted to Dr. L. A. Walker of Helena, Montana, for this gland.

We have, however, the same criterion to apply here as in the parasitic amoebae of the bone marrow, namely, the morphology of the nucleus at mitosis and the number of chromosomes. We have accordingly made an intensive search of these sections, and have found among the supposed amoebae more than ten instances of some phase of the mitotic process.

The amoebae are often distinguished by slight differences in the stainability of the cytoplasm. In some instances, there is a suggestion of deterioration, but not in all. A normal amoeba with vacuolated protoplasm, pseudopodia, and small spherical nucleus with central spherical karyosome and heavy peripheral rim of chromatin is shown in figures 1 and 2. A deeply stained, clouded nucleus closely resembling those often found in amoebae in sections of intestinal wall stained in iron haematoxylin is shown in figure 3.

The appearance of such amoeboid cells at mitosis is shown in figure 4. The nucleus of this amoeba is in the late anaphase, the chromosomes have reached the ends of the elongated nucleus, where their individuality is somewhat masked by overlapping and fusion.

If we apply to this nucleus the same criterions which were applied in the preceding article to dividing amoebae in the bone marrow, we find that this nucleus meets these tests. It has, in the first place, an intact nuclear membrane which has persisted to a late phase of mitosis. In the second place, there is a meridional intradesmose on the inner face of the nuclear membrane connecting the two poles of the nucleus. In the third place, the number of chromosomes is neither 24 nor 48, the numbers reported for human cells. It is apparently not over 6. It is impossible to be certain of the full number because of the overlapping of these small bodies.

We therefore conclude that these amoeboid cells are not human cells, and that they are parasitic amoebae. In view of their similarity in the active state and in mitosis to *Endamoeba dysenteriae* and also because of the coexistence of the intestinal infection by this amoeba in this specific case, we infer that it is this same infection that has in this instance reached the inguinal gland. This conclusion opens the possibility that Hodgkin's disease may be amoebiasis of the lymphatic system and calls for investigation along other than morphological lines.

LITERATURE CITED IN THE TWO ARTICLES

Ely, L. W.
1920. The second great type of chronic arthritis. A laboratory and clinical study. Arch. of Surgery, **7**, 158–183, 21 figs. in text.

Ely, L. W., Reed, A. C., and Wyckoff, H. A.
1922. The amoeba as the cause of the second great type of chronic arthritis. California State Jour. Med., **20**, 59.

Evans, H. M.
1918. Unpublished work cited by Babcock, E. B., and Clausen, R. E., *in* "Genetics in Relation to Agriculture" (McGraw-Hill Book Co., New York and London), p. 538.
1921. Unpublished work cited by Sharp, L. W., *in* "An Introduction to Cytology" (McGraw-Hill Book Co., New York), p. 362.

Guyer, M. F.
1910. Accessory chromosomes in man. Biol. Bull., **19**, 219–234, pl. 1.
1914. Accessory chromosomes in man. Science, n.s., **39**, 721–722; 941–942.

Kofoid, C. A.
1920. Hookworm and amoebiasis in California. California State Jour. Med., **18**, 329–332.

Kofoid, C. A., and Swezy, O.
1921. On the free, encysted, and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine. Univ. Calif. Publ. Zool., **20**, 169–198, pls. 18–22, 3 figs. in text.
1922. On the occurrence of *Endamoeba dysenteriae* in the bone lesions in *arthritis deformans*. California State Jour. Med., **20**, 59.

Kofoid, C. A., Swezy, O., and Boyers, L. M.
1922. On the coexistence of Hodgkin's disease and amoebiasis. Jour. Am. Med. Assoc., **78**, 532.

Montgomery, T. H.
1912. Human spermatogenesis, spermatocytes and spermiogenesis; a study of inheritance. Jour Acad. Nat. Sci. of Philadelphia, **15**, 1–22, pls. 1–4.

Painter, T. S.
1921. The Y-chromosome in mammals. Science, n.s., **53**, 503–504.

Reed, Dorothy M.
1902. On the pathological changes in Hodgkin's disease, with especial reference to its relation to tuberculosis. The Johns Hopkins Hospital Rep., **10**, 133–196, pls. 6, 7.

Swezy, O.
1922. On mitosis in *Endamoeba coli*. Univ. Calif. Publ. Zool., **20** (*in press*).

Wieman, H. L.
1917. The chromosomes of human spermatocytes. Amer. Jour. Anat., **21**, 1–22, pls. 1–4.

Winiwarter, H. von.
1912. Études sur la spermatogenese humaine. I. Cellule de Sertoli. II. Heterochromosome et mitoses de l'epithelium seminal. Arch. d. Biol., **27**, 91–189, pls. 6–7.

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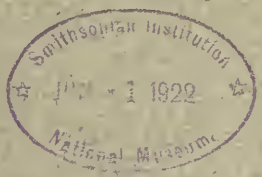
MITOSIS IN THE ENCYSTED STAGES OF ENDAMOEBA COLI (LOESCH)

BY

OLIVE SWEZY



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MITOSIS IN THE ENCYSTED STAGES OF ENDAMOEBA COLI (LOESCH)

BY
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CONTENTS

PAGE
Introduction ... 313
Acknowledgments ... 315
Morphology ... 315
Mitosis ... 317
Prophase ... 317
Metaphase ... 319
Anaphase ... 320
Telophase ... 320
Discussion ... 321
Summary ... 325
Literature cited ... 325
Explanation of plates ... 328

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INTRODUCTION

A complete study of the various phases of the life history of the intestinal amoebae of man is confronted by several difficulties which, thus far, have proved almost insurmountable. One of these is the difficulty of culturing these parasites outside of the human body. It is true that cultures have been made which yielded an abundance of amoebae (for example, Yoshida, 1920), but, in most cases at least, either the amoebae have not been the true intestinal species, or they have shown undoubted evidences of degeneration which render them unsuitable for further investigation, either cytological or developmental. In the latter case, the amoebae are possibly only those which survived from the original inoculation in a more or less degenerate condition.

Another difficulty is correlated with the change in habitat of the cysts of these organisms on discharge in the stool. If the seat of infection is the upper part of the colon where the conditions differ somewhat from those prevailing in the rectum and stool, such differences undoubtedly react on these delicate organisms so that only those protected by a fully developed cyst may pass out of the body without showing some of the deleterious effects of this great environmental change. Such changes as those incident to passing from the region of the mucosa to the lumen and thence downward in the stool appear to be associated with the process of encystment. It is also possible that changes in the temperature of the environment or in available oxygen may temporarily suspend or accelerate developmental changes within the organism, such as mitosis, until a more favorable location is reached, when normal development may again proceed.

It is evident to any one who works over amoebic cysts that a varying number of the cysts ordinarily met with in stool examinations show the results of degenerative changes, particularly if the specimen has remained in the laboratory some time before examination. In any consideration of the various stages of the life history, these forms must be eliminated or the results will be disastrous from a cytological standpoint. It is also doubtful how much value can be placed on the cytological study of the amoebae which have been inoculated into kittens, since an alien host may tend to produce some morphological changes.

Endamoeba coli is a common infection of man but is rarely present in great numbers in human stools. The different stages of mitosis are seen only occasionally and are rare in the cysts in most stools. It is therefore difficult to make a sufficient number of observations of mitosis in the cysts of this human intestinal amoeba to use as a basis for the investigation of this process.

The material on which this study was made, and from which the figures illustrating it were mainly drawn, was found in a single stool from an overseas ex-soldier in the University Infirmary. It occurred among more than one thousand stools containing infections of *E. coli* which were examined among more than six thousand routine examinations with a total of four thousand two hundred persons. A fresh stool was available and many of the cysts were found with mitosis in progress. An attempt was made to secure further development by keeping the stool in a warm oven and making slides at intervals of a few hours. In the first set made, the slides showed two and four

nuclei in the majority of the cysts; those made later contained practically no cysts with dividing nuclei but a relatively greater number of eight-nucleated cysts. This would seem to indicate that division proceeded normally in this case. The only good mitotic figures were found on the first set of slides, with the exception of that shown in figure 23, plate 31, which was taken from a set of slides made after the stool had been standing in the laboratory for twenty-four hours. The fact, however, that this single stool was the only one among several thousands which gave an abundance of division forms indicates the rarity of the occurrence of mitosis in cysts in stools examined in the ordinary manner. The fortunate coincidence of discharge during a phase of normal nuclear multiplication and prompt access to the material and its early fixation has made this study possible.

ACKNOWLEDGMENTS

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MORPHOLOGY

The active amoeboid stages of *Endamoeba coli* may be readily distinguished from *Councilmania lafleuri*, in both fresh and stained material, by granular pseudopods and lack of clearly marked ectoplasm, as well as by more sluggish movements in the living forms. The cysts are distinguished on careful examination of the karyosome when unstained or in iodine-eosin, and may be more readily differentiated in fixed and stained preparations. These differences have been summed up in a recent paper (Kofoid and Swezy, 1921) and need not be repeated here, except to point out the fact that they may, in some cysts, be almost entirely obscured during certain stages of the process of mitosis, such as the early prophase and late telophase, when chromosomes cannot be counted in the encysted amoebae. The same fact will probably hold true of division in the active forms of these two species, but, thus far, division in the active amoebae has not been found in our material. More data are needed on this point.

In the following paragraphs a brief description of the encysted forms only of *Endamoeba coli* will be given, since this study is confined to the process of mitosis in that phase only of its life history.

The cysts of *Endamoeba coli* vary from 10 to 25μ in diameter. Larger cysts than these have been reported in the literature but they may have been the cysts of *Councilmania lafleuri*, and not of *Endamoeba coli*.

In unstained material in fresh physiological salt solution, the cysts appear homogenous, the highly refractive cyst wall standing out sharply against the material of the stool. In cysts that are moribund the nuclei become visible, sometimes appearing with great clearness even under low powers of the microscope. In preparations stained with iodine-eosin the contained structures may usually be readily seen, including the nuclei, the chromatoidal bodies, and the glycogen mass, if the latter be present. The nuclei show the typical excentric karyosome, and chromatin granules on the membrane. In permanent preparations stained with iron-haematoxylin or other stains, a few scattered granules may be observed on the linin reticulum which fills the nuclear spaces.

Uninucleate cysts have been exceedingly rare in the material under observation, and in nearly every case have presented a prophase stage of mitosis (pl. 29, fig. 1). In the binucleate and quadrinucleate cysts the majority, in the stool above referred to, showed some phase of the mitotic process, but occasional individuals have been found with the nuclei in the ordinary resting condition (pl. 29, fig. 2). These show the typical excentric karyosome, chromatin granules on the nuclear membrane, and a few scattered granules on the linin reticulum. The greater part of the cyst in the binucleate phase is usually occupied by a single, large, centrally located glycogen vacuole or by several smaller vacuoles, forcing the two nuclei out to the periphery. The remaining space is often closely packed with acicular, splinter-like, chromatoidal bodies (pl. 29, fig. 2). These vary in quantity in different cysts but are almost always present in the binucleate and quadrinucleate ones, sometimes in such abundance that detection of the nuclei becomes an impossibility, particularly in the various mitotic phases. In later stages the chromatoidal material generally becomes greatly reduced in quantity, either being massed in a few large clumps (pl. 31, fig. 25) or appearing as slender splinters (pl. 31, fig. 28). Occasionally a few black, rounded-up granules are the only vestiges of chromatoidal material remaining in the cyst (pl. 31, fig. 26).

At the beginning of encystment the cytoplasm generally contains a large amount of glycogen. This may be so great in extent as to give a dense brownish color to the entire cyst in iodine-eosin stain, or it may be localized in small spheres with outlines more or less sharply defined. With the process of fixation the glycogen forms a single mass or several large masses whose locations, in stained preparations, appear as large, clearly defined vacuoles (pl. 29, figs. 3–7), the glycogen itself having been dissolved in the aqueous solutions in the process of staining.

MITOSIS

Binary fission of *Endamoeba coli* in its active form has not thus far been described by any investigator. It is probable that it occurs mainly in the upper part of the colon and occupies such a short time that individuals undergoing this process do not reach the exterior before its completion. Binucleate free individuals may sometimes be present (Dobell, 1919), but no clue to their further history has been found.

In the following paragraphs, an outline of the process of mitosis in the encysted form of *Endomoeba coli* will be given. Some minor gaps in this process have yet to be filled, but a sufficient number of stages are here presented to indicate the line of development throughout.

PROPHASE

The prophase of mitosis is marked by a considerable increase in the amount of chromatin contained within the nucleus. This is relatively small in the normal trophozoite and is distributed in typical fashion, with chromatin granules attached to the nuclear membrane, and a small, spheroidal excentric karyosome (pl. 29, fig. 2). A few chromatin granules may also be found on the linin reticulum which fills the nucleus. At the approach of the prophase the linin network disappears and the karyosome becomes lost in the mass of chromatin found in the center of the nucleus (pl. 29, fig. 1), usually distributed in irregular masses and granules. At the same time the nucleus becomes elongated and the larger masses of chromatin become distributed as numerous small granules filling more or less of the interior (fig. 3). This is the stage most abundant in amoebae containing two nuclei. In fact, most individuals of the two- and four-cell stages in *Endamoeba coli* in our material seem to be in the early prophase.

In the one- and two-cell stages, when many chromatoidal bodies, as well as the nuclei, fill the narrow space between the glycogen mass and the periphery, the nuclei are exceedingly difficult to differentiate, particularly if the chromatin granules have left their position on the nuclear membrane. In a few cases only have the cysts been devoid, or nearly so, of chromatoidal material (pl. 29, figs. 6, 7). The nuclei are often obscured also by the change in the staining reactions of the chromatin that may be noticed at this stage, in its failure to take a dark stain with iron-haematoxylin or in its ease in destaining. Frequently, as the chromatin gathers at the center of the nucleus, it stains less intensely with iron haematoxylin, appearing as a grey mass with a few darker staining granules within it (pl. 29, fig. 3). Later the central mass takes an intensely dark stain and at the same time shows a considerable increase in volume (pl. 29, fig. 4).

It is impossible to say whether any chromatin substance is extruded from the nucleus, owing to the amount of darkly staining material found in the extranuclear regions. There is no evidence of an exchange of material in either direction, though it may well take place. It is equally impossible, however, to consider the abundant chromatoidal bodies as formed by nuclear extrusions alone, as they are often present in great quantity and size before division begins (pl. 29, fig. 1). Their abundance in the earlier stages of mitosis and their gradually lessening quantity as mitosis proceeds, as is the case also with the glycogen, would seem to indicate that both these substances serve as reserve food materials that are gradually used up in the great metabolic activity required by the repeated processes of mitotic division. The glycogen is formed before the chromatoidal bodies appear, and it likewise is absorbed before the latter are. Cysts containing more than four nuclei very rarely show any traces of glycogen, while the presence of chromatoidal bodies is frequently noted in such cysts.

About the time the chromatin granules have all disappeared from the nuclear membrane, the chromatin in the central mass has assumed the appearance of small, oblong granules, or rods, without definite number or arrangement, but scattered through the major portion of the interior of the nucleus (pl. 29, figs. 4, 5). A definite spireme or skein was not observed, nor could the formation of the chromosomes be followed in detail. The mass of granules becomes less in amount, either by condensation or other metabolic change, and the double chromosomes appear (pl. 29, fig. 7, lower nucleus).

The polar masses or daughter centrosomes are probably formed from a part of the original karyosome but it has not been possible to trace this throughout the changes in general massing of the chromatin in the central part of the nucleus. As the polar masses divide and move to opposite poles, a slender thread, the intradesmose, is drawn out between the two portions and lies at all times within the nuclear membrane. This is the same intranuclear structure as that found in *Councilmania lafleuri* which we called *intradesmose* (Kofoid and Swezy, 1921) to distinguish it from the *paradesmose* of the flagellates, the latter structure being outside of the nuclear membrane (Kofoid and Swezy, 1915).

Along with the formation of the chromosomes, the nucleus becomes elongated (pl. 29, figs. 4–7). The spindle is formed between the polar masses within the nuclear membrane which persists throughout the entire process of division. In the equatorial plate stage, the spindle fibers, to which the chromosomes are attached, are found to lie in the outer surface of a double cone (pl. 30, fig. 13). The intradesmose is found to lie close to the spindle fibers (pl. 29, figs. 8, 9; pl. 30, fig. 15), and, as it soon loses its intense staining reactions, it becomes difficult to differentiate from the spindle fibers. It is probably retracted in the late telophase along with the drawn-out nuclear membrane.

The first division of the nucleus was not found in the material under observation, but it is probable that the mode of procedure is the same in this as in the later divisions. In cysts containing more than one nucleus the time of division of each is not always synchronous for the different nuclei (pl. 29, fig. 7; pl. 31, figs. 19–23), which accounts for the frequent appearance of individuals of *Endamoeba coli* with five, six or seven nuclei, or any number between eight and sixteen.

METAPHASE

There is some suggestion that the chromosomes divide early in the prophase, as they appear double before the spindle is formed (pl. 29, fig. 7). No conclusions regarding the relative positions of the chromosomes at this stage, or as to how they become attached to the spindle fibers, could be drawn from observation on the material studied. When the metaphase is reached the appearance of division in the chromosomes is lost, and they are attached to the middle of the spindle as twelve slightly irregular, compact, rod-like granules. In end view these form a ring (pl. 30, fig. 13) in the middle plane of the nucleus with a clear area in the center of the spindle.

ANAPHASE

The anaphase is probably of longer duration than is the metaphase, judging by inference from the relative number of individuals of both stages observed. In fact, the majority of dividing nuclei found in the material were in this stage, the next in abundance being the early prophase.

In most cases, there seems to be little difference in the size and shape of the chromosomes during the anaphase, although occasionally one or two chromosomes may be somewhat smaller in size than the others (pl. 30, figs. 10, 13; pl. 31, fig. 28). The chromosomes range in size from .5μ to 1.5μ.

As the division of the chromosomes seems to occur before the spindle is formed (lower nucleus in figure 7, plate 29), the separation in the anaphase probably follows the original split, as in *Giardia* (Boeck, 1919), and *Trichonympha* (Kofoid and Swezy, 1919). The separation of the two moieties is not synchronous for the six chromosomes, but considerable variation is found between them in almost every case examined. In many nuclei the variations in this respect are striking, some of the chromosomes being well on their way toward the poles before the final parting of the last daughter chromosomes is completed (pl. 30, figs. 10, 11, 15; pl. 31, fig. 19).

As the chromosomes divide they move toward the poles, as the spindle fibers shorten (pl. 30, fig. 15). Along with this change the nucleus begins to elongate somewhat, becoming less rotund through its middle and gradually assuming a dumb-bell shape (pl. 30, fig. 16).

TELOPHASE

With the migration of the chromosomes to the poles they soon lose their identity and become massed together (pl. 30, fig. 16; pls. 31, figs. 19–21). This process is accompanied by an elongation of the nucleus and its constriction near the middle. The latter change proceeds until the nucleus has become a long, slender dumb-bell (pl. 31, fig. 21), which may be drawn out until the sister nuclei come to lie on opposite sides of the cyst. The nucleus parts in the middle, the drawn-out points are retracted and two new spherical nuclei are formed (pl. 30, fig. 17).

The complete reconstruction of the nucleus evidently takes place before the prophase of the next division is entered upon (pl. 30, fig. 18). Corresponding steps in the process of mitosis of the nuclei seem

to be closely similar, if not identical, in the two-, four-, and eight-cell stages. There is considerable decrease in actual size of the nuclei with each successive division (pl. 31, fig. 23), giving to the cysts with eight or more nuclei a greater disproportion between the size of the cyst and that of the nuclei (pl. 31, fig. 22) than may be found in the earlier stages, where the nuclei are relatively large (pl. 29, fig. 2). Thus in the one- and two-cell stage the diameter of the nucleus may be equal to one-third that of the cyst, while in the eight-cell stage the diameter of the nucleus may be one-sixth, or even less than that, of the cyst.

Division of the protoplasmic body has not been observed in *Endamoeba coli,* either in the active or in the encysted amoeba. In the latter case it may well be that it is delayed until after leaving the body of the host and entering a new one. Figure 25, plate 31, would suggest that the multinucleate somatella is released from the cyst before dividing into the individual amoebas, though this may be the result of repeated divisions in the active amoeba without plasmotomy intervening, as individuals with two or more nuclei may occasionally be observed.

DISCUSSION

The literature on the subject of division in the parasitic amoebae is filled with confusion and misinterpretations, the result, largely, of generalizations on too scanty data. The earlier conceptions of this process as a "simple amitosis" were, perhaps, unavoidable, but with the constantly growing knowledge of the types of mitosis that may be found in the Protozoa at the present time, modifications of these earlier conclusions are to be expected as the result of further investigations along this line. It seems very probable that simple amitosis is as rare in the higher Protozoa as in the Metazoa.

In Dobell's recent discussion (1919) of the nuclear behavior of the human intestinal amoebae, he gives a number of division figures of *Endamoeba dysenteriae,* taken from active amoebae from intestinal ulcers of cats experimentally infected with human amoebae. The type of division shown in these figures differs somewhat from that shown herewith for *Endamoeba coli,* and it may be that the alien environment which the human amoebae find in the cat has produced some modifications which are not present when the amoebae occupy their normal habitat, or that division in the active form differs from

that in the cyst. In *Endamoeba coli* Dobell found a typical spindle in the first nuclear division of the encysted amoeba, but he also states that "the subsequent nuclear divisions are all similar, and resemble those of *Endamoeba histolytica*." Of the latter amoeba he says, "I do not consider the nuclear division of *Endamoeba histolytica* to be a regular mitosis. On the other hand, I cannot call it an amitosis. It seems rather to belong to an intermediate category." Certain resemblances may be found in his figure 64, plate 4, to some which are presented in this paper.

In *Councilmania lafleuri* (Kofoid and Swezy, 1921) the type of mitosis is closely similar to that shown herewith for *Endamoeba coli*, both having polar masses or centrosomes and an intradesmose. *Councilmania lafleuri* has, however, eight chromosomes, differing in this respect from *Endamoeba coli* with its six chromosomes.

The nuclear division of the active amoeboid phase of *Endamoeba coli* has not as yet been observed by any investigator. Free amoebae with two nuclei are occasionally met with and in one case (pl. 31, fig. 25) one with eight nuclei was seen. These are not common, however, and it is probable that binary fission is the general rule among the active amoebae, and that it occurs before the organisms leave the upper part of the large intestine. The individual shown in figure 25, plate 31, may be one that has escaped from its cyst without dividing, but it is also possible that such somatellas are sometimes formed without encystment as well as in the cysts, as in the case of the parasitic flagellates, *Giardia* (Kofoid and Christiansen, 1915) and *Trichomonas* (Kofoid and Swezy, 1915).

The question of a division center or centrosome in the parasitic amoebae is one about which conflicting opinions may be found in the literature on the subject. Dobell (1919) found no structures which he could regard as centrosomes in *Endamoeba dysenteriae*. In a brief description of the division of *Endamoeba dysenteriae*, Mathis and Mercier (1916) find, however, that such structures are present. In 1917 the same authors figure division in *Endamoeba legeri* from *Macacus* as a mitosis with centrosome, spindle, and centrodesmose (= intradesmose). The centrosome originates in the karyosome and, as it divides, the two daughter centrosomes draw out between them a slender, darkly staining thread, which we have called the intradesmose. The spindle fibers are formed between the centrosomes within the nuclear membrane (their figs. II, b-d, h). This method of procedure is closely similar to that outlined above for *Endamoeba*

coli. The prophase of the two amoebae seems also to be similar, as their figure III-e differs from the same stage in *Endamoeba coli* only in the lack of characteristic chromatoidal bodies.

The abundance of chromatoidal substances present in the early prophase of *Endamoeba coli* obscures much of the development in that period, the individuals from which the present studies were made being especially rich in that substance. The lack of this substance in *Endamoeba legeri* makes that species a more favorable object for study of these early stages.

The polar masses or centrosomes in *Endamoeba coli* are rather large (spread out) and often diffuse (pl. 29, fig. 8, 9), or may be small (pl. 30, fig. 13). In the telophase they are incorporated with the chromosomes (pl. 30, figs. 15, 16) but their further history could not be traced with certainty. It is probable that the centrosome forms the karyosome in the new nucleus, while the chromatin of the chromosomes becomes wholly, or in large part, distributed over the linin reticulum and nuclear membrane.

It is possible that a definitive centrosome may be present within the larger structure which forms the polar mass, a suggestion of which is found in the fact that this may be very small in some individuals.

The intradesmose is formed when the centrosome or karyosome divides, and connects them until the final parting in the telophase. The origin of this structure is shown with greater clearness in the figures of *Endamoeba legeri* by Mathis and Mercier (1917), referred to above, and is called by them the centrodesmose. It is often difficult to differentiate the intradesmose from the spindle fibers, hence its absence in some of our figures.

The differences to be found between the type of division described above for *Endamoeba coli* and those described by other workers for the various species of non-parasitic amoeba consist largely in the structure of the spindle, centrosome, and intradesmose and in the number and size of the chromosomes. The differences, however, are more apparent than real, as a closer examination will show. The residual mass of chromatin derived from the karyosome and forming the polar caps and central spindle (intradesmose) is greater in amount in *Naegleria gruberi* (Wilson, 1916) and *Amoeba tachypodia* (Gläser, 1912), than in *Endamoeba coli* or *Endamoeba legeri,* but the behavior of the structures is essentially the same in both groups. The division of *Amoeba hyalina,* as described by Hartmann and Chagas (1910), repeats the same process in its essential details, although the amount of chromatin involved in the centrosomes is here quite minute.

The division of these amoebae differs from that of the parasitic flagellates in that the centrosomes and intradesmose are found within the nuclear membrane, the homologue of the latter structure, the paradesmose, and the centrosome of the flagellates always being outside of the nuclear membrane, the relation of the centrosome to the flagella in the flagellate making the extranuclear position an essential one for these structures in that group.

In a recently published paper Yoshida (1920) describes a process of autogamy in *Endamoeba coli* in which two, four, or even six, nuclei may fuse to form a "syncaryon." On the basis of his experiments, he claims that "the daughter-nuclei in the cyst stage correspond exactly to the gamete nuclei of other Protozoa," but gives no cytological evidence of gamete formation. As a further step in the process of autogamy he finds that the amoebae develop numerous vacuoles and their nuclei shrink and degenerate until they finally burst. The phenomenon he has described is one occurring occasionally in parasitic amoebae when undergoing degeneration outside of their normal host, and has no known relation to sexual or ordinary developmental processes. The process of "autogamy" was first described for *Endamoeba coli* by Schaudinn (1903) and later figured by Hartmann (1909) from descriptions in the earlier paper. Some of these figures are similar to those shown on plate 29, of the one- and two-nucleate amoebae with the large glycogen vacuole filling the center. However, instead of adding the third stage, a four-nucleate amoeba, the first stage, or a *Blastocystis* was again arbitrarily used as that resulting from "autogamy" in the two-nucleate cyst. Along with these stages Schaudinn added other phases of the yeast-like *Blastocystis* frequently found in human faeces (Kofoid, 1921). This process has since been figured in textbooks as the normal sexual process in amoebae. It has been repeated in textbooks again and again for amoebae but thus far no satisfactory cytological evidence has been brought forward to support such an interpretation for the ordinary steps in nuclear division (pl. 29, figs. 1–7), or for the degeneration phases of amoebae that have been placed in an alien environment.

SUMMARY

Nuclear division in *Endamoeba coli* is a simple mitosis which takes place within the nuclear membrane.

The chromosomes are probably six in number and divide before the spindle is formed.

Polar masses or centrosomes are formed by division of a chromatin body probably derived from the karyosome. An intradesmose connects these bodies as they separate and continues to unite them until the nuclei part in the telophase.

The centrosomes, spindle, and intradesmose are contained within the nuclear membrane, which is present throughout the entire process of mitosis.

Three successive mitotic divisions take place, resulting in the production of eight nucleated cysts. A fourth division may also occur.

LITERATURE CITED

Boeck, W. C.

1919. Studies on *Giardia microti*. Univ. Calif. Publ. Zool., **19**, 85–136, pl. 1, 19 figs. in text.

Dobell, C.

1914. Cytological studies on three species of amoebae—*A. lacertae* Hartmann, *A. glebae* n. sp., *A. fluvialis* n. sp. Arch. f. Prot., **34**, 139–189, pls. 7–11.

1919. The amoebae living in man (London, John Bale, Sons & Danielsson). Pp. vi + 155, 5 pls.

Gläser, H.

1912. Untersuchungen über die Teilung einiger Amöben, zugleich ein Beitrag zur Phylogenie des Centrosoms. Arch. f. Prot., **25**, 27–152, pls. 3–8, 5 figs. in text.

Hartmann, M.

1909. Autogamie bei Protisten und ihre Bedeutung für des Befruchtungsproblem. *Ibid.*, **14**, 264–334, 27 figs. in text.

Hartmann, M. and Chagas, C.

1910. Ueber die Kernteilung von *Amoeba hyalina* Dang. Mem. Inst. Oswaldo Cruz, **2**, 64–126, pls. 4–9, 3 figs. in text.

Kofoid, C. A.

1921. A critical discussion of the chromidial formation of nuclei, of autogamy, and of the multiple division of polyenergid nuclei, with special reference to the unity of the mechanism of heredity in the Protozoa and Metazoa. Anat. Rec., **20**, 223–225.

Kofoid, C. A., and Christiansen, E. B.
1915. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–54, pls. 5–8, 1 fig. in text.

Kofoid, C. A., and Swezy, O.
1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad. Arts Sci., **51**, 289–278, pls. 1–8, **7** figs. in text.
1919. Studies on the parasites of the termites. III. On *Trichonympha campanula* sp. nov. Univ. Calif. Publ. Zool., **20**, 41–98, pls. 5–12, 4 figs. in text.
1921. On the free, encysted, and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine. *Ibid.*, **20**, 169–198, pls. 18–22, 3 figs. in text.

Mathis, C., and Mercier, L.
1916. La division simple chez *Entamoeba dysenteriae*. C. R. Soc. Biol., Paris, **79**, 982–984.
1917. Affinités d'*Entamoeba legeri* Mathis et d'*E. coli*. Arch. Zool. Exp. et Gen., **56**, 63–72, 4 figs. in text.

Schaudinn, F.
1903. Untersuchungen über die Fortpflanzung einiger Rhizopoden. Arb. Kais. Gesundh., **19**, 547–576.

Wilson, C. W.
1916. On the life-history of a soil amoeba. Univ. Calif. Publ. Zool., **16**, 241–292, pls. 18–23.

Yoshida, K.
1920. The encystment of dysentery amoebae *in vitro*. Jour. Exper. Med., **28**, 387–413, pls. 30–43.

EXPLANATION OF PLATES

All drawings are of *Endamoeba coli* and were made with camera lucida from preparations fixed in Schaudinn's fluid and stained with iron haematoxylin. Magnification, × 2500.

PLATE 29

Fig. 1. Mononucleate cyst with nucleus in the prophase and the cytoplasm filled with typical chromatoidal bodies.

Fig. 2. Binucleate cyst with nuclei in the resting phase.

Fig. 3. Binucleate cyst with nuclei in early prophase.

Fig. 4. A later prophase with the chromatin leaving the nuclear membrane.

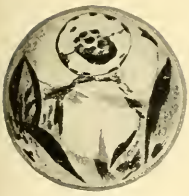
Fig. 5. Prophase with the nuclear membrane entirely free from chromatin.

Fig. 6. Prophase.

Fig. 7. Late prophase with the spindle formed in one nucleus and not yet begun in the other. Note evidence for splitting of the chromosomes in lower nucleus.

Fig. 8. Anaphase. Note abundance of chromidial bodies in cytoplasm.

Fig. 9. Same stage as that of figure 8.



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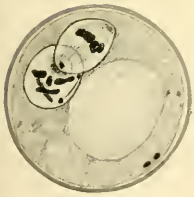

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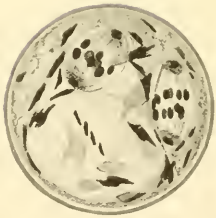

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PLATE 30

Fig. 10. Early anaphase or late metaphase. Some chromosomes in each nucleus not yet divided.

Fig. 11. Anaphase with one chromosome not yet divided.

Fig. 12. Anaphase.

Fig. 13. One nucleus in metaphase—seen from one pole—and the other in early anaphase. Large glycogen vacuole absent.

Fig. 14. Anaphase.

Fig. 15. Late anaphase.

Fig. 16. Telophase. Chromosomes have begun to lose their individuality.

Fig. 17. Telophase. Note lack of synchronism in division of nuclei.

Fig. 18. Reorganization nearly completed in the four nuclei.

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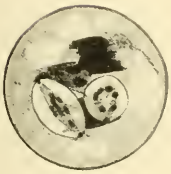

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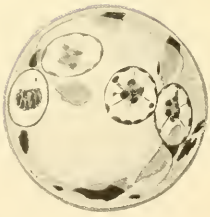

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PLATE 31

Fig. 19. Third division of nuclei. Each of the four nuclei shows a different phase.

Fig. 20. Telophase. Beginning of constriction of the nuclei.

Fig. 21. Later telophase. Glycogen vacuole absent in these stages.

Fig. 22. Completion of third division.

Fig. 23. Early prophase of third division.

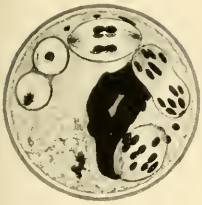
Fig. 24. Fourth division on its way.

Fig. 25. Active amoeba with eight nuclei.

Fig. 26. Active binucleate amoeba with huge glycogen vacuole.

Fig. 27. Eight-nucleate cyst with chromatoidal substance almost absorbed.

Fig. 28. Eight-nucleate cyst with typical, splinter-like chromatoidal bodies.



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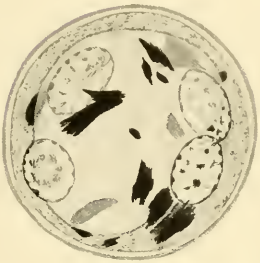

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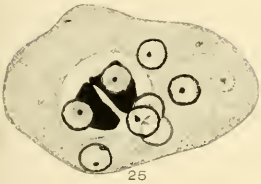

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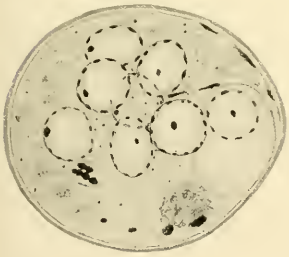

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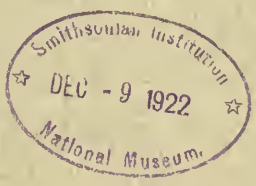
THE NEUROMOTOR APPARATUS OF PARAMAECIUM

BY

CHARLES WILLIAM REES



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CONTENTS

	PAGE
Introduction	334
Acknowledgments	334
Review of literature	334
Micro-injection	336
Technique and Methods	336
Culture	336
Technique	336
Morphology	338
Brief description of *Paramaecium*	338
Neuromotor apparatus	338
Neuromotor center	340
Peripheral fibers	341
Fibers of the cytostome and cytopharynx	342
The neuromotor system during division	344
The neuromotor apparatus of *Paramaecium* compared with that of the flagellates and with other ciliates	345
Experimental	346
Micro-injection of vital dyes	346
Intravitam staining	346
Axial gradients	347
Micro-dissection	349
Discussion	350
Summary	351
Literature cited	353
Explanation of plates	356

INTRODUCTION

Paramaccium, because of its universal occurrence in aquaria and the ease with which it may be cultured, has long been a favorite organism for use in class instruction and laboratory investigation. It is well adapted for this study because it is a generalized rather than a highly specialized type. For the same reason a description of the system of neuromotor fibers in *Paramaecium,* a system equalling in complexity the nervous systems of many of the lower Metazoa, will be of general interest.

Acknowledgments

The present work was conducted during the past two years at the University of California under the direction of Dr. Charles A. Kofoid, and to his profound insight most of the discoveries were due. Dr. Charles V. Taylor rendered invaluable assistance in explaining and demonstrating micro-injection and micro-dissection equipment. The fixing, staining, imbedding, and sectioning were directed by Dr. Olive Swezy, whose expert advice in the use of Heidenhain's iron haematoxylin made possible the wonderfully clear differentiation of the neuromotor fibrils. Professor A. B. Domonoske, of the Department of Mechanical Engineering, Mr. H. N. Cooper, Curator of the Department of Physical Chemistry, and Mr. W. J. Cummings also gave much appreciated assistance on technical matters of apparatus.

Review of the Literature

A review of most of the literature on conductile fibers in the Protozoa was written by Dr. Taylor (1920). The only addition made here is a discussion of some of the papers pertaining particularly to *Paramaecium.*

In 1902 Neresheimer made attempts to stain nerve elements in *Paramaecium, Spirostomum,* and *Stentor coeruleus* with Mallory's triple stain. He found certain myonemes in *Stentor* and *Spirostomum* but failed to find any in *Paramaecium.* He concluded, therefore, that none were present and set about to prove this by the following method: He treated both *Paramaecium* and *Stentor* with equally

dilute solutions of narcotics, e.g., morphine, strychnine, caffein, curare, and nicotine, and found *Stentor* to be easily narcotized, while *Paramaecium* remained apparently normal.

Khainsky (1910) made a contribution to the morphology of *Paramaecium*, the importance of which he did not realize. He fixed the animals with concentrated bromine water and found that none of the trichocysts was discharged, whereas they are frequently discharged in other fixatives. In his plate 1, figures 6 to 8, he shows distinct fibers connected to the basal granules of the cilia. He comments as follows: "Die Gleichartigkeit der Fibrillen mit dem übrigen Cilienteile begeist dass ihre Hauptrolle in der Ernährung und dem Wachstum der Cilie besteht."

Most of the recent work on contractile and conductile elements of protozoans has been done by the University of California Zoological Laboratory. The name neuromotor apparatus was given by Kofoid (1915) to the integrating fibrillar complex in *Giardia* associated with the blepharoplasts, parabasal bodies, and other active organelles of locomotion. The literature is already extensive, comprising 473 printed pages and 42 plates in fifteen special research papers.

A neuromotor apparatus was described by Sharp (1915) in *Diplodinium ecaudatum*, a very complex member of the order Oligotricha. Parts of this system had been previously described (Braun, 1914), but either a supporting or contractile function had been ascribed to it. Yocom (1918) described a similar system in the hypotrichan *Euplotes*. These two ciliates, the one a parasite, the other a free-living form, possess neuromotor systems easily comparable. In each the system consists of a motorium from which fibers extend to the motor organelles and also to the membranelles surrounding the cytostome. The motorium is in no way connected with the nucleus. Taylor (1920), by the method of micro-dissection, demonstrated that the system in *Euplotes* possesses conductile functions. His conclusions are based on three hundred and fifteen experiments on which extensive notes were taken. His conclusions were as follows:

> These experimental evidences do not support the assumption that the fibrillar system in *Euplotes* is either contractile or supporting in function, but they indicate that this complex system of fibers does possess conductive properties functioning in the coördination of the movements of the locomotor organelles with which it is intimately associated (Taylor, 1920).

Micro-injection

The successful search for a neuromotor apparatus in *Paramaecium* was the outgrowth of micro-injection studies (Rees, 1922). To accomplish micro-injection, it was found necessary to isolate the organisms in rounded drops, so that they would not be disturbed by the forces of surface tension (Rees, 1922). This led to a search for a method of making stained preparations of undistorted animals, which was accomplished by fixing them in centrifuge tubes instead of applying the fixative after they had been attached to the slide by means of egg albumen.

TECHNIQUE AND METHODS

Culture

The animals for this work were obtained from wild stock that had been brought in with débris from the creek bed and cultured in covered battery jars. After being injected or otherwise operated upon, they usually thrived if given plenty of food. The importance of food was demonstrated only after the death of many organisms within three or four hours after the operation. The practice had been to place them in small drops of culture fluid from which other individuals had been removed by coarse filter paper. Even the controls did not survive twenty-four hours under such conditions.

A careful search was first made for some toxic substance. It was thought that volatile poisons were present in the vaseline used to seal down the covers or that sufficient of the salts of mercury were formed in the injecting and isolating instruments to prove fatal. After weeks of investigation, a rich autoclaved and fully ripened hay infusion was substituted for the filtered culture fluid. The result was astonishing. The animal survived after the operation and grew rapidly, often dividing twice within twenty-four hours.

Technique

Contrary to the statement of Neresheimer (1902), a fibrillar system may be differentiated in *Paramaecium* with Mallory's triple connective tissue stain, even when the animals are attached to the slide coated with egg albumen. They were fixed in Zenker's fluid

which was dropped on the slide while hot. The same processes of staining were employed as were used by Sharp (1917) and Yocom (1918). The system appears as a network of fine anastomosing fibrils in the ectoplasm.

Another method of staining with Mallory's was as follows: The organisms were killed and fixed either in Zenker's fluid or picro-mercuric (Yocom, 1918) solution in centrifuge tubes. They were placed by means of a pipette in a small piece of folded silk bolting cloth. In this they were run through the stains and alcohols to xylol, then transferred to a Lefevre watch glass and concentrated in the groove by means of a camel's hair brush. A drop of balsam was added and they were placed on the slide by means of a pipette. The surface fibrils did not show in animals so stained, but clear views of the cytostomal and cytopharyngeal systems were obtained.

Iron alum haematoxylin was by far the best stain. Schaudinn's solution used hot was the best fixer. The animals nearly always discharged their trichocysts. Picro-mercuric used hot sometimes fixed the animals with trichocysts intact, but they did not stain so well as when Schaudinn's fluid was used. The fact that trichocysts are discharged in Schaudinn's is fortunate for whole mounts, as otherwise the dorsal and ventral whorls of fibrils would not be made visible. In only four of over twenty trials was success attained with whole mounts, the difficult part of the process being the destaining, which required from two to four hours with cold 2 per cent iron alum. Sometimes the animals turned yellow or became opaque and had to be discarded. When destaining goes on as is desired, the organisms change color from black to blue and then to bluish gray, and the protoplasm is always quite clear. The end of the destaining process is reached when they are quite gray with the nucleus still quite dark. Only faint outlines of the neuromotor system can be seen at this time even with oil immersion. The whorls of ramifying fibrils become clearly differentiated only after clearing in xylol and balsam.

Infiltration and imbedding were carried out at first in gelatine capsules as described by Metcalf (1908), but later it was found that glass capsules made of tubing by sealing one end in the flame were more satisfactory.

As will be shown by an examination of the plates, the thick sections (15μ) were very valuable because they gave more extensive views of the cytopharyngeal fibers than did the thin ones. The lines of basal granules and trichocysts were most clearly seen in unstained

living animals isolated in small drops, but after having been discovered there they were found in whole mounts stained with both Mallory's and iron haematoxylin. The lines of basal granules were also brought out when the animals were killed in 10 per cent formalin and fixed twenty-four hours in the dark in Von Rath's osmic acid picro-mercuric fixative.

When animals were killed in 1 per cent strychnine solution, the cytoplasm, with the included trichocysts, contracted to a small sphere, leaving the transparent pellicle in its original position. The trichocysts were then discharged and looked like astral rays protruding from the cytoplasm. The clearest views of the ciliary lines in the pellicle were then obtained. Wonderfully clear views of trichocysts and basal granules were also obtained when animals killed in concentrated bromine water were placed on a slide and a little aniline blue solution run under the cover.

MORPHOLOGY

Brief Description of Paramaecium

In describing *Paramaecium*, the terms oral and aboral will be used instead of dorsal and ventral. Otherwise, the terminology is the some as that used for bilaterally symmetrical animals.

Paramaecium was well described by Ellis (1769) as "an animal with a longish body and a groove in one end like a gimlet." The cytostome, a slit-like aperture, extends from the caudal end of this groove and lies in the posterior two-thirds of the body. It is continued as the cytopharynx which runs obliquely caudad to the vicinity of the posterior contractile vacuole. There are two contractile vacuoles, one anterior and one posterior, which pulsate rhythmically about twenty times per minute. The macronucleus is oblong, deeply staining about one-third as long as the body, and its posterior end lies near the anterior end of the cytostome. The micronucleus commonly lies in a little groove in the macronucleus. The cytoplasm contains numerous food vacuoles in continual cyclosis.

Neuromotor Apparatus

In this work, studies have been made of the outer endoplasm and the ectoplasm and pellicle. The ectoplasmic structures include the cytostome and cytopharynx, also longitudinal rows of cilia, and trichocysts which we have found to be arranged in whorls. The

membranelles of the cytopharynx and the motor and defensive organelles covering the surface of the body have been found to be connected by an integrated fibrillar system which has its center in the outer endoplasm at the anterior end of the cytostome.

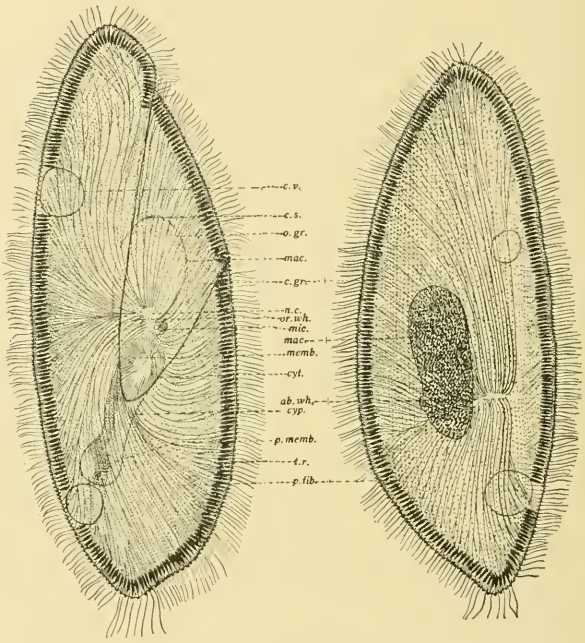
Maupas (1883), Bütschli (1887–89), Kölsch (1902), Maier (1903), Schuberg (1907), and Khainsky (1910) have described the pellicle of *Paramaecium*. All are agreed that the cilia arise from depressions in the surface, while the Indian club-shaped trichocysts pierce deeply into the ectoplasm and reach the surface of papillae arranged in a definite pattern. The fact that the ciliary and trichocyst lines are not parallel was also discovered. They were described by Bütschli (1889) as "rechtwinklig gekreuzt." Maupas describes the pellicle as a pattern of rhombohedral plates, the cilia being in the center, the trichocysts in the corners of these plates. Bütschli, Kölsch, and Maier thought the plates were hexagonal. Schuberg found rhombohedrons, hexagons, and parallelograms. Khainsky's observations confirm those of Schuberg.

But it is only when one makes maps of the entire pellicle that the true relationships of these various patterns are revealed and the idea of grooves and ridges rather than depressions and papillae is obtained. The terminology employed by Kofoid and Swezy (1919) to describe *Trichonympha campanula* is here employed to describe *Paramaecium*. In *Trichonympha* the cilia arise from the ridges, in *Paramaecium* they arise from the grooves, but in both animals they are arranged in longitudinal rows. On the aboral surface of the latter they are almost parallel; on the oral side, slightly oblique and the rows from opposite sides meet in a series of V's. The apices of these V's lie in a line that runs longitudinally through the cytostome and hence obliquely through the oral surface from the anterior to the posterior end. This is the ciliary suture (fig. A, *c.s.*, also pl. 35, fig. 26, and pl. 36, fig. 30).

The ridges differ from those of *Trichonympha* in that they are not parallel, but are arranged with reference to the neuromotor system in whorls (figs. A and B, also pl. 35, figs. 27, 28, and pl. 36, figs. 31, 33). For this reason, they are cut across at regular intervals by the grooves so that they appear as papillae which constitute interrupted ridges. It is the intercrossing of these whorls of trichocysts and rows of cilia that gives the various rhombohedral, hexagonal, and parallelogrammic patterns previously described. The courses of the whorls being the same as those of the neuromotor fibers, it will not be necessary to describe them separately.

Neuromotor Center

All the fibers of the neuromotor system ramify out from the neuromotor center (fig. A, *n.c.*) which is just anterior to the cytostome near the oral surface. In plate 35, figure 22, are seen two bodies to which fibers converge. This figure was drawn from a



A B

Fig. A. Semi-diagrammatic sketch of *Paramaecium caudatum*, oral view, showing oral whorl of peripheral fibers and the ciliary lines, ciliary suture, and trichocyst ridges.

Fig. B. Aboral view of *Paramaecium caudatum*, showing aboral whorl, ciliary lines, and trichocyst ridges. Abbreviations: *c. v.*, contractile vacuole; *c. s.*, ciliary suture; *o. gr.*, oral groove; *mac.*, macronucleus; *c. gr.*, ciliary grooves; *n. c.*, neuromotor center; *or. wh.*, oral whorl; *mic.*, micronucleus; *memb.*, cytopharynx membranelle; *cyt.*, cytostome; *cyp.*, cytopharynx; *t. r.*, trichocyst ridges; *p. fib.*, peripheral fibers.

Figures A and B are reprinted by permission of the editor of the American Naturalist from Rees (1921).

section fifteen microns thick which was fixed in picro-mercuric, stained twelve hours in haematoxylin, and destained until almost white. A small body to which the cytostomal fibers converge may also be seen in well destained whole mounts. The fact that all the fibers of the system converge toward a point in this region suggests a motorium: a body that functions as a coördinating center or in some way provides a contact of fibers effecting a basis for such interconnection and coördination.

Peripheral Fibers

For convenience the neuromotor system will be described under the two headings, peripheral fibers, and cytopharyngeal fibers. The peripheral fibers are arranged in two whorls, one on the oral, the other on the aboral side (figs. A and B, *or. wh., ab. wh.;* pl. 32, figs. 1–6, pl. 34, figs. 13–19).

The oral whorl is the more extensive, covering the entire oral side and extending, on the left, over part of the aboral side. The fibers lie very near the surface, impinging upon the endoplasm as they approach the neuromotor center. They are gray when stained with iron haematoxylin, and red when stained with Mallory's. The thickness of the individual fibers has not been determined but they appear very fine when seen under a magnification of 1330 diameters. In the oral groove when followed from their diverging ends in the ectoplasm toward the neuromotor center, their course is obliquely caudad to the margin of the cytostome, where they turn and converge obliquely cephalad. Over the remainder of the surface, they run in gracefully curved lines directly toward the neuromotor center. The anastomosing ends in the ectoplasm are very numerous and profusely branched, forming a thick fibrillar complex (figs. A and B; pl. 32, figs. 1, 3, 4; pl. 34, figs. 13–19).

The fibers of the aboral whorl converge, forming a large V-shaped figure with the apex on the left, opposite but slightly posterior to the cytostome (fig. B; pl. 32, figs. 2, 3, 4). Here they appear to dip into the endoplasm in their course toward the neuromotor center. The ramifications of the fiber ends of this whorl resemble, in every respect, those of the aboral whorl.

The meeting places of the fibers of the oral and aboral whorls in the ectoplasm cannot be made out very distinctly, because, in order to see them, the animals must lie either on their right or their left

sides. Being thicker through, in this direction, too much of the light is shut out. It will be noted that the oral whorl converges from both sides toward the "center," while the aboral whorl converges from only the right side. For this reason, the meeting place on the right side of the animal is formed of diverging fiber ends (pl. 32, fig. 4). On the left the fibers of the oral whorl meet the converging apex of the aboral whorl (pl. 32, fig. 2). Thus the entire ectoplasm is supplied with a thick complex of diverging fibers.

Actual connections of fibers to the basal granules are figured in plate 33, figures 7–12, and plate 35, figures 21, 25. Kahinsky (1910) confirms the presence of these fibers, but calls them ciliary rootlets. They can be made out clearly only in thin sections (not over 2.5 microns).

The facts that in whole mounts and thick sections the neuromotor fibers can be traced to the immediate vicinity of the basal granules and that in thin sections actual connections can be seen are submitted as proof that the ciliary rootlets represent the connections of the neuromotor fibers with the basal granules.

The figures cited above also show fine fibers connected to the inner ends of the trichocysts. These also are believed to be neuromotor fibers innervating the defensive organelles of the body.

Fibers of the Cytostome and Cytopharynx

The oral groove (fig. A, *or. gr.*) extends from the anterior end of the animal to the region of the posterior two-thirds of the body. As previously described, the peripheral fibers, only, underlie the surface of this groove. They exhibit no special modification in this region.

The cytostome is an elongated oval aperture extending obliquely caudad from the posterior margin of the oral groove (fig. A. *cyt.*). Around its margin is a row of peristomal cilia. Two fibers connect the basal granules of these cilia with the neuromotor center. They run one in each margin and are continued posteriorly into the cytopharynx.

The cytopharynx begins at the anterior margin of the cytostome. From this point to the posterior cytostomal margin it is a V-shaped depression (pl. 33, fig. 8). Then it becomes a subcylindrical structure except at the extreme posterior end, where there is an enlargement. The enlargement is not perfectly spheroidal (pl. 33, fig. 11), but is broadly indented on the oral side. The posterior extremity of the

cytopharynx is near the posterior contractile vacuole and ends abruptly at the endoplasm where the food vacuoles form.

There are two membranelle zones in the cytopharynx, one of which, the anterior, was described (Maier, 1903) as an undulating membrane. But careful studies of cross as well as longitudinal sections and also of the living animal lead us to believe that this zone is made up of a series of flat, paint-brush-like membranelles rather than connected longitudinal rows of cilia such as would constitute an undulating membrane (pl. 33, figs. 9, 10; pl. 35, figs. 21, 23).

The anterior zone extends from the beginning of the cytopharynx to the enlargement. Except in the posterior region, the cilia of each membranelle are of about equal length, but at the enlargement each brush becomes progressively longer.

A fan of heavy fibers, each of which is of about twice the diameter of a peripheral fiber, runs from the neuromotor center directly to the anterior membranelle zone of the cytopharynx. Some of these fibers may be seen in the living unstained animals as well as in most whole mounts and cross and longitudinal sections. One gets an exceptionally good view in thick well destained longitudinal sections (pl. 35, fig. 22).

Two of the fibers, on the right margin of the fan, are heavier than the others. In thin cross-sections of the cytopharynx a structure made up of basal granules is seen resembling a basal plate (pl. 33, fig. 10). Maier (1902) describes this plate but does not mention the fibers. The two heavy fibers above mentioned run along the margins of each plate. All the fibers of the fan are about equidistant, almost parallel, and do not branch. They have not been traced positively beyond the anterior membranelle zone.

The posterior membranelle zone is less regular in outline than the anterior, and less extensive. It was clearly seen first in a section fifteen microns thick stained with Mallory's. This zone begins as a series of narrow, rounded, paint-brush membranelles just anterior to the enlargement on the left side of the cytopharynx. Farther posteriorly in the enlargement it spreads out on the indented surface, the basal granules of its cilia being in the oral side (pl. 33, fig. 11; pl. 35, figs. 21, 23). So far as is known, this posterior membranelle zone has not been previously described.

Two fibers were noted above (page 342) running from the neuromotor center in each margin of the cytostome to the basal granules of the peristomal cilia and on into the cytopharynx. They run into the posterior membranelle zone (pl. 35, fig. 26) and branch profusely even into the endoplasm beyond it.

The neuromotor system of *Paramaecium*, therefore, as has been demonstrated by the foregoing description of the neuromotor center and the peripheral and cytopharyngeal fiber systems, is, from a morphological point of view, a structurally adapted, coördinating mechanism. It is easily compared with a metazoan nervous system, being composed of fibers that connect the organelles of locomotion, nutrition, and defense with a definite center.

The Neuromotor System during Division

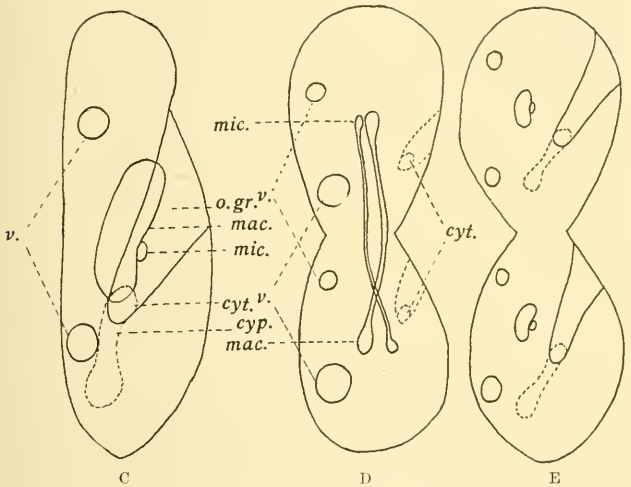
One is surprised in reading the literature of *Paramaecium* to find only two accounts of the fate of the old cytostome and cytopharynx in dividing animals and of the origin of new ones. One of these is quoted by Doflein (1916) from R. Hertwig (1890). The old cytostome is represented as budding off a new one which becomes the cytostome of the posterior daughter.

The second account is by Child (1916) who finds that the old cytostome and cytopharynx are dedifferentiated and disappear. Jennings (1908) had previously observed the same process, also the occurrence of a slight shortening of the body in animals about to divide. The new cytostome and cytopharynx of the posterior daughter are redifferentiated in the position occupied by the old cytostome. The cytostome and cytopharynx of the anterior daughter are entirely new structures (figs. C, D, E).

The accuracy of this account I verified with only a 16-millimeter objective and 20*x* oculars when animals from a rapidly dividing culture were transferred to a Syracuse watch glass. Dividing animals usually remain attached to some débris in the bottom of the dish during the entire process. In one case observed, the entire process from the time division became manifest to the separation of the two daughter animals required forty-five minutes.

A complete account of the changes in the neuromotor apparatus during the division process cannot as yet be given. It is certain that the cytostomal and cytopharyngeal fibers of the anterior daughter are new structures, as they appear very soon after the constriction (pl. 34, fig. 20). Since the origin of a new cytostome gives rise to a new center, it is likewise certain that the peripheral fibers of the anterior daughter are to a very large extent newly formed. But since the fibers, other than those of the cytopharynx, were not seen in the stained animals in division, the extent to which dedifferentiation and redifferentiation occurred in the peripheral fibers of the posterior daughter has not been ascertained.

The evidence does not indicate that the neuromotor fibers take part in mitosis or division. It indicates rather that the behavior is very similar to that in *Euplotes* (Yocom, 1918). In the latter, however, the cytostome of the mother animal marks the place of origin of the cytostome of the anterior rather than the posterior daughter. The difference here is no doubt accounted for in the posterior location of the cytostome of *Paramaecium*.



Figs. C, D, E. Three views to show fate of old cytostome, cytopharynx, and contractile vacuoles, and the origin of new structures in the two animals. Slightly modified after Child. Abbreviations: *v.*, contractile vacuoles of undivided animals; *v. l.*, new contractile vacuoles; *mic.*, micronucleus; *o. gr.*, oral groove; *mac.*, macronucleus; *cyt.*, cytostome; *cyp.*, cytopharynx.

The Neuromotor Apparatus of Paramaecium Compared with that of the Flagellates and with other Ciliates

The neuromotor fibers of flagellates are connected with the centroblepharoplast complex which functions in mitosis.

Trichonympha is a flagellate covered with ciliahke flagella (Kofoid and Swezy, 1919) which are connected to the blepharoplast by a network of oblique fibers. In *Paramaecium* the cilia are also connected to a definite center by fibers. It is significant that in these two

unrelated organisms whose life habits are very different similar conductile elements should be evolved. The fundamental difference is that so far as is known in *Paramaecium* the neuromotor system does not function in mitosis and division and is not a part of the mitotic apparatus, as in flagellates.

The system in *Paramaecium* resembles in many ways that in *Diplodinium* (Sharp, 1914) and *Euplotes* (Yocom, 1920). In all three animals a set of fibers runs from a center to the cytostomal and cytopharyngeal organelles and also to the organelles of locomotion.

Paramaecium is unique among the animals thus far described in possessing an innervated system of defensive structures, the trichocysts. It is to be regarded as a generalized member of the ciliate group. The fibers are more numerous and finer and not organized into tracts. The coördinating center is less highly developed and harder to differentiate.

EXPERIMENTAL

Three different experimental methods were utilized in attempting to demonstrate that the fibrillar system of *Paramaecium* is conductile.

Micro-Injection of Vital Dyes

Micro-injection of vital dyes was used to determine whether the fibers take the same stains as do nerve fibers in the Metazoa. Child's method of determining the axial gradient was employed (Child, 1916) to find whether or not this gradient in *Paramaecium* is related to the physiological organization that should exist in an animal with a complex fibrillar system whose function is to receive and transmit stimuli. The third method is that of micro-dissection similar to the experiments of Taylor (1920) on *Euplotes patella.*

Intra Vitam Staining

Wilson (1910) summarized a method of staining nerves *intra vitam* in the Metazoa with Grübler's methylene blue. The stain was injected into the living tissue very soon after the death of the animal. In about twenty minutes to two hours the nerve fibers were stained. The tissues were then fixed in saturated ammonium picrate or 7 per cent ammonium molybdate and dehydrated quickly in 100 per cent alcohol or glycerine.

To demonstrate the presence of nerve fibers in *Paramaecium* by modifications of this method, a one-tenth of one per cent solution of the stain was injected into the living animals. They were then examined under oil immersion almost continuously for periods varying up to four hours. As long as they lived, the ectoplasmic structures did not stain. The endoplasmic inclusions stained light green. At death, the animals became opaque, so that fibers could not be seen.

Paramaecium was also placed over-night in a one-four-hundredth per cent solution of the stain. As nearly as could be determined the results were not different from those where micro-injection was practiced.

Axial Gradients

Alcohol and other narcotics, e.g., morphine hydrochlorate 1 per cent, antipyrin 1 per cent, nicotine $\frac{1}{10}$ per cent, and strychnine 1 per cent were used rather than potassium cyanide in determining the axial gradient (Child, 1916) for two reasons: (1) The animals are slowed down in the narcotics so that they may be more carefully observed. This is not the case with potassium cyanide. (2) Neresheimer (1902) used the above narcotics, and I desired to check on his experiments, from which he concluded that *Paramaecium* does not behave in these solutions as do animals with nervous systems.

According to the conception of gradients, the physiologically anterior end of an animal does not necessarily coincide with the morphologically anterior end, but the former is the region having the highest metabolic rate. Tashiro demonstrated that a nerve fiber has a higher metabolic rate than other structures of protoplasm. He also proved that the gradient in an efferent nerve is from the "center" toward the end organ. In an afferent nerve, this gradient is reversed (Tashiro, 1917).

From the foregoing, it is clear that if the fibrillar system in *Paramaecium* is conductile, the physiologically anterior end should be the region of the neuromotor center just anterior to the cytostome. But we should also expect the end morphologically anterior to possess a high metabolic rate because of the presence in this sensitive region of so many fibers which conduct afferent impulses. The avoiding reaction in particular (Jennings, 1906) results from the transmission of contact stimuli from this anterior region and the resulting coördinated movements of the neuromotor organs, causing the animal to reverse its direction and then to swim forward in a different course.

In one respect, the experiments were disappointing. *Paramaecium* did not disintegrate in the manner of planarians and annelid worms. Instead blisters appeared invariably over the posterior contractile vacuole. Sometimes the animals died without further distortion. At other times, the blister grew larger and larger until the ectoplasm was disrupted and the endoplasm flowed out. For this reason, the metabolic rate of the region of the neuromotor center was not determined.

But it was found that the anterior cilia invariably ceased beating first, from five to fifteen seconds earlier than the cilia of the cytopharynx and posterior end. The following is a typical experiment.

EXPERIMENT NUMBER 6

11:50 A.M. Animal isolated in 6 per cent alcohol to 20 c.c. of which a small drop of 1 per cent methylene blue had been added.
11:52 A.M. Circus movements to the left.
11:56 A.M. Movements slower, slight blister over posterior contractile vacuole.
12 M. End over end movements very slow. Blister much larger.
12:05 P.M. Anterior cilia cease.
8 seconds later. Cytopharyngeal cilia cease.
2 seconds later. Posterior cilia cease.

When 4 per cent alcohol was used, the process often required thirty minutes, but the events were in every case similar to those above described.

The animals lived in 1 per cent antipyrin about twelve minutes, in $\frac{1}{10}$ per cent nicotine about twenty minutes, in 1 per cent morphine hydrochlorate about seventeen minutes, in 1 per cent strychnine about five seconds. Except in the strychnine, which was used too concentrated, the behavior was similar to that observed in alcohol.

The experiments show that there is a gradient in *Paramaecium* of a nature that one would expect if the fibers are conductile in function.

The experiments certainly do not support Neresheimer's contention that the organisms cannot be narcotized. The movements of *Paramaecium* are appreciably slowed down in all the narcotics used. In one case an animal remained quiet in 4 per cent alcohol for fifteen minutes. On being disturbed, it moved about very slowly, in sharp contrast to the rapid movements of an animal not so treated.

The behavior of *Paramaecium* in these solutions of narcotics was found to be not unlike that of annelid worms, except that *Paramaecium* did not similarly disintegrate.

Micro-Dissection

It is difficult to demonstrate by the method of micro-dissection that the fibers in *Paramaecium* are conductile because:

In the first place, the animal is not highly specialized, as is the case with *Euplotes* and as the locomotor organelles are smaller, their coördinated movements are harder to see.

Secondly, the pellicle is exceedingly hard to cut. It gives way under the pressure of the needle point without being severed and returns to its normal position when the pressure is released. *Euplotes* was found to be much easier to dissect because of its greater resistance to the needle. Furthermore, the only fibers clearly visible in the unstained living animal are those of the cytopharynx.

Partial transsections through the cytopharynx and hence through these fibers were made, partial transsections also being made elsewhere as control experiments. In addition to the above cuts, the region of the neuromotor center was cut and otherwise injured with the needle point.

Short, rapidly tapering needles made of pyrex rods were most effective. With a needle of this type, it was found possible to cut animals in two.

In four cases where it could be positively determined that the cytopharyngeal fibers had been cut, there was seen a marked difference in rate and amplitude of vibration between the membranelles anterior to the cut and posterior to it. The movement of the posterior membranelles was slower and through a smaller amplitude than that of the anterior membranelles. Severe injury, on both sides of the membranelle zone, produced no detectable disturbance so long as the cytopharyngeal fibers remained intact. Transsections elsewhere likewise had no effect on the action of the membranelles. In one instance, a wide cut, not quite reaching the cytopharyngeal fibers, was made in the left side of the animal. No effect was detected in the beating of the membranelles except in cases where these fibers were injured. Verworn (1889) records similar experiments with *Spirostomum*.

When animals were isolated in gelatine, it was possible to observe the ciliary vibrations very clearly. In three cases, severe injury to the animal in the region of the neuromotor center caused a pronounced disturbance of ciliary movement. In one case, no less than four distinct groups of cilia were observed, two on each side, each moving independently of the others. Those on one side were vibrating in

opposite directions to those on the other. On the other hand, severe injury to other parts of the body than the motorium did not affect the coördinated movement of the cilia.

Jennings and Jamieson (1902) found that pieces of *Paramaecium* and other ciliates behave not unlike intact animals, except that in the smaller pieces the movements are more feeble. These observations I easily confirmed in the case of *Paramaecium*. In a small piece from an animal isolated in gelatine not over one-fifth as large as the intact organism, the cilia continued to vibrate normally for at least five minutes. Five such cases were observed, three in anterior pieces and two in posterior pieces. In no case, however, were the cilia observed to reverse their movements in these small pieces. Intact animals in gelatine reverse the movements as a rule every few seconds. In all cases, however, all the cilia of the piece beat in unison.

The evidence indicates that, while the cilia may beat in the absence of neuromotor impulses from the neuromotor center, this center does exert an influence through the neuromotor fibers in coördinating and integrating the direction of vibration of the cilia as a whole.

DISCUSSION

As intimated by earlier writers (Taylor, 1920), the main objections to the idea of conductility in fibrillar systems of the Protozoa come from advocates of the cell theory who fail to recognize that complexity of structure may be attained within a single cell and without cell division and division of labor among its products. The nucleus has been the main object of study of those who have investigated Protozoa.

But no one will deny that the protoplasm of *Paramaecium* conducts stimuli. Else the complex behavior exhibited in the avoiding reaction (Jennings, 1901) would be impossible. This being the case, the structures best adapted to conduct stimuli are those of the neuromotor system.

Sharp (1914) developed a clever argument in discussing the neuromotor apparatus of *Diplodinium*. He ascribed to the fibers one of three functions, that of support, of contractility, or of conductivity. Then, by the method of elimination, he showed that the fibers are conductile.

If we investigate the fibrillar system of *Paramaecium* with the same ideas in mind, we get a more convincing proof than in the case of *Diplodinium* and *Euplotes*. The frailness of the fine fibers precludes the idea that they are skeletal or supporting. It is a familiar fact that *Paramaecium* under a variety of conditions adverse to its normal functioning contracts into a spheroidal body. But, clearly, to get a sphere of an elongated animal the longitudinal axis must be shortened and the transverse axes lengthened. If this change of shape were produced by the neuromotor fibers, those whose direction is transverse would elongate while those whose direction is longitudinal would have to contract. In other words, they would have to function similarly to antagonistic sets of muscles which function in response to nervous impulses. Such coördinated functioning of these extremely fine fibers without innervation is unlikely.

The morphology also suggests that the fibers are conductile. Their fine branches, which converge from every part of the animal to the motorium, resemble very closely similar branches in metazoan nervous systems. Their connections with the basal granules and trichocysts and with a center suggest these conductile and coördinative functions even more strongly. The set that runs in the cytostome and cytopharynx connecting with the same center also suggests coördination. When stained with Mallory's triple connective tissue stain, they take the acid fuchsin. This also suggests that the fibers resemble chemically the nerve fibrils of metazoans.

The experimental evidence, while less conclusive, is still important in several particulars. The gradient that could be demonstrated was of the type to be expected on the basis of neuromotor function. The transsections of the cytopharyngeal fibers and resultant lack of coördinated movement of the membranelles are evidence which shows that at least the cytopharyngeal fibers function in the transmission of impulses.

SUMMARY

1. A fibrillar system may be differentiated in *Paramaecium* with Mallory's triple connective tissue stain. The fibers are very fine and branch profusely. They take the acid fuchsin and are therefore red by transmitted light.

2. By means of Heidenhain's iron haematoxylin this very complex system of fibers has been differentiated both in whole mounts and sections.

3. The cilia arise in longitudinal grooves in the pellicle. These grooves are slightly oblique on the oral side and meet in the ciliary suture, a line that runs obliquely through the oral surface from the anterior to the posterior end.

4. The neuromotor system consists of two peripheral whorls of fibers, the larger on the oral side, the smaller on the aboral side, and of two other groups, the cytopharyngeal fibers, one running to the anterior zone of membranelles in the cytopharynx, the other around the margin of the cytostome to the posterior membranelle zone. This zone has not been previously described. All the fibers of the peripheral whorls and of the cytopharynx ramify out from the neuromotor center at the anterior end of the cytostome.

5. This is a body at the anterior end of the cytostome corresponding to the motorium of other ciliates. This body is differentiated with difficulty.

6. The old cytostome and cytopharynx and, so far as is known, the neuromotor system also, are dedifferentiated prior to division. The cytostome, cytopharynx, and neuromotor fibers of the anterior daughter are new structures. The extent to which dedifferentiation and redifferentiation occur has not been determined.

7. The neuromotor system of *Paramaecium* is of a generalized type in conformity with the lack of specialization of parts in the organism.

8. Grübler's methylene blue, when injected into the animals, did not stain the neuromotor fibers.

9. The axial gradient in *Paramaecium* could not be fully determined because the animals did not disintegrate. There is, however, an anterio-posterior gradient in cessation of ciliary action along the longitudinal axis which would be expected if the fibrillar system is interpreted as conductile.

10. A distinct interruption of coördinated movements is effected when the region of the neuromotor center is injured. Cutting the cytopharyngeal fibers interrupted the coördination of movement between the membranelles anterior to the cut and those posterior to it.

11. The morphological as well as the experimental studies indicate that the fibrillar system of *Paramaecium* is conductile in function.

LITERATURE CITED

Braune, R.

1914. Untersuchungen über die im Wiederkäuermagen vorkommenden Protozoen. Arch. Prot., **32**, 112–170, pls. 3–6.

Bütschli, O.

1887–89. Bronn's Klassen und Ordnungen des Tierreichs (Protozoa), **1**, viii + 2035 pp., pls. 1–79.

Child, C. M.

1915. Senescence and rejuvenescence (Chicago, Univ. Chicago Press). xi + 481 pp., 201 figs. in text.

Doflein, F.

1916. Lehrbuch der Protozoenkunde. Eine Darstellung der Naturgeschichte der Protozoen mit besonderer Berücksichtigung der parasitischen und pathogenen Formen. (Jena, Fischer). Ed. 4, xv + 1190 pp., 1198 figs. in text.

Ehrenberg, C. G.

1830. Infusionsthierchen als vollkommene Organismen. Ein Blick in das tiefere organische Leben der Natur (Leipzig, Leopold Voss). xviii + 547 pp., pls. 1–64.

Ellis, J.

1769. Observations on a particular manner of increase in the animalculae of vegetable infusions with the discovery of an indiffusible salt arising from hemp-seed put into water until it becomes putrid. Roy. Soc. London. Phil. Trans., **59**, 138–152, pl. 1.

Hertwig, R.

1890. Ueber die Konjugation der Infusorien. Abh. K. Bay. Akad. Wiss., **17**, 1–83, pls. 1–4.

Jennings, H. S.

1906. Behavior of the lower organisms (New York, Columbia Univ. Press). xiv + 355 pp., 144 figs. in text.

1908. Heredity variation and evolution in Protozoa. Proc. Am. Phil. Soc., **47**, 393–546, 5 figs. in text.

Jennings, H. S., and Jamieson, C.

1902. Studies on reactions to stimuli in unicellular organisms. The movements and reactions of pieces of ciliate Infusoria. Biol. Bull., **3**, 225–234, 4 figs. in text.

Khainsky, A.

1910. Zür Morphologie und Physiologie einiger Infusorien (*Paramaecium caudatum*) auf Grund einer neuen histologischen Methode. Arch. Prot., **21**, 1–6, pls. 1–3, 2 figs. in text.

Kofoid, C. A., and Swezy, O.

1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Am. Acad. Arts and Sci., Boston, **51**, 289–378, pls. 1–8, 7 figs. in text.

1919. Studies on the parasites of the termites. I. On *Streblomastix strix*, a polymastigote flagellate with a linear plasmodial phase. II. On *Trichomitus termitidis*, a polymastigote flagellate with a highly developed neuromotor system. III. On *Trichonympha campanula* sp. nov. IV. On *Leidyopsis sphaerica*, gen. nov., sp. nov. Univ. Calif. Publ. Zool., **20**, 1–116, pls. 1–14, 8 figs. in text.

Kölsch, K.

1902. Untersuchungen über die Zerflussungserscheinungen der ciliaten Infusorien. Zool. Jahrb., Abt. Anat. u. Ontol. der Tiere, **16**, 273–422, pls. 26–28, 5 figs. in text.

Maier, H. N.

1903. Über den feineren Ban der Wimperapparate der Infusorieu. Arch. Prot., **2**, 305–324, pl. 7, 1 fig. in text.

Maupas, E.

1883. Contribution à l'étude morphologique et anatomique des infusoires ciliés. Arch. Zool. exper. gén., (2), **1**, 427–664, pls. 19–24.

Metcalf, M. M.

1908. Opalina, its anatomy and reproduction with a description of infection experiments and a chronological review of the literature. Arch. Prot., **13**, 195–378, pls. 14–28, 17 figs. in text.

Nereshfimer, E. R.

1903. Die Höhe histologischen Differenzierung bei heterotrichen Ciliaten. Arch. Prot., **2**, 305–324, pl. 7, 1 fig. in text.

Rees, C. W.

1921. The neuromotor apparatus of *Paramecium*. Am. Nat., **55**, 464–468, 2 figs. in text.

1922. The micro-injection of *Paramaecium*. Univ. Calif. Publ. Zool., **20**, 235–242.

1922. The isolation of *Paramaecium* with notes on the technique of micro-injection and micro-dissection. (Manuscript.)

Sharp, R. G.

1914. *Diplodinium ecaudatum* with an account of its neuromotor apparatus. Univ. Calif. Publ. Zool., **13**, 42–122, pls. 3–7, 4 figs. in text.

Schuberg, A.

1905. Über Cilien und Trichocysten einiger Infusorien. Arch. Prot., **6**, 61–110, pls. 4–5.

Swezy, O.

1915. Binary and multiple fission in *Hexamitus*. Univ. Calif. Publ. Zool., **16**, 71–88, pls. 9–11.

Tashiro, S.

1917. A chemical sign of life (Chicago, Univ. of Chicago Press). vii + 142 pp., 5 figs in text.

Taylor, C. V.

1920. Demonstration of the function of the neuromotor apparatus in *Euplotes* by the method of micro-dissection. Univ. Calif. Publ. Zool., **19**, 403–470, pls. 29–33, 2 figs. in text.

1920. An accurately controllable micropipette. Science, n.s., **51**, 617–618, 1 fig. in text.

Verworn, M.

1889. Psychophysiologische Protistenstudien (Jena, Fischer). viii + 219 pp., pls. 1–6, 27 figs. in text.

Wilson, J. G.

1910. Intra-vitam staining with methylene blue. Anat. Rec., **4**, 267–277.

Yocom, H. B.

1918. The neuromotor apparatus in *Euplotes patella*. Univ. Calif. Publ. Zool., **18**, 337–396, pls. 14–16, 1 fig. in text.

EXPLANATION OF PLATES

All figures are of *Paramaecium*, drawn with camera lucida and, unless otherwise stated, stained with Heidenhain's iron haematoxylin.

PLATE 32

Fig. 1. Oral view showing the oral groove, cytostome, and oral whorl of peripheral fibers ramifying out from the neuromotor center. × 356.

Fig. 2. View of left side, showing meeting points of the anastamosing ends of the fibers of the oral whorl with those of the aboral whorl. × 356.

Fig. 3. Aboral view showing the aboral whorl. × 356.

Fig. 4. View of left side. The anastamosing ends of the fibers of the oral whorl meet the converging apex of the aboral whorl. × 356.

Fig. 5. Slightly oblique section, 5μ thick, through the cytostome, showing converging fibers of the oral whorl in the outer endoplasm. × 670.

Fig. 6. Oblique section through the cytopharynx, showing some of the fibers of the oral whorl and also of the aboral whorl. × 670.

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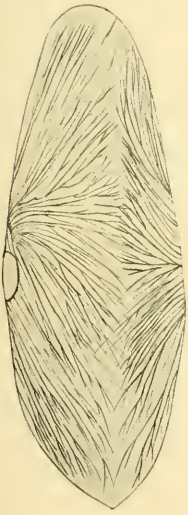

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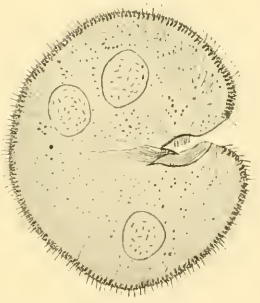

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PLATE 33

All figures of cross-sections through the same organism. 2.5μ thick. $\times$ 670.

Fig. 7. Oral groove at the beginning of the cytostome and cytopharynx, showing ends of peripheral fibers connected with the basal granules and trichocysts.

Fig. 8. Through the mid-cytostome region, showing membranelle brush of anterior membranelle zone.

Fig. 9. Through the posterior cytostome region and the cytopharynx, showing membranelle brush of anterior membranelle zone.

Fig. 10. Through cytopharynx, showing membranelle brush of anterior zone and beginning of posterior membranelle zone.

Fig. 11. Through indented portion of cytopharynx and posterior membranelle zone.

Fig. 12. Through extreme posterior end of cytopharynx, showing cilia of posterior membranelle zone partly obscured by ingested food bodies.

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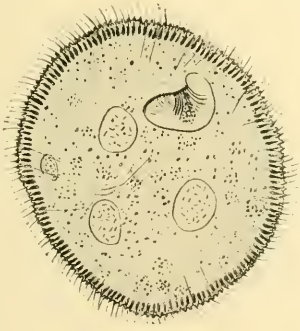

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PLATE 34

Figs. 13–19 are successive longitudinal sections, 10μ thick, through the same organism. × 365.

Fig. 13. Through left side, showing peripheral fibers of oral whorl.

Fig. 14. Showing fibers of oral whorl and also of aboral whorl.

Fig. 15. Showing fibers of both oral and aboral whorls and section of macronucleus.

Fig. 16. Fibers of both oral and aboral and margin of cytostome.

Fig. 17. Through the cytostome and cytopharynx, showing fibers of aboral whorl and a few fibers of oral whorl.

Fig. 18. Section through right side of macronucleus.

Fig. 19. Through right side of animal. Fibers of both whorls in periphery.

Fig. 20. Whole mount of dividing organism, showing macronucleus, the constriction, and in each daughter organism a new cytostome, cytopharynx, and cytopharyngeal fibers. Mallory's triple connective tissue stain. × 356.

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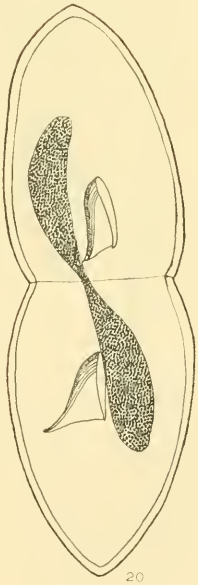
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PLATE 35

Fig. 21. Longitudinal section, 15μ thick, through the cytopharynx, showing anterior and posterior membranelle zones and indentation of cytopharynx. Mallory's triple connective tissue stain. × 670.

Fig. 22. Longitudinal section, 15μ thick, through the cytopharynx, showing fibers of cytopharynx running from a deeply staining body and peripheral fibers ramifying out from another body.

Fig. 23. Longitudinal section, 15μ thick, through cytostome and cytopharynx, showing anterior membranelle zone, part of posterior membranelle zone and peripheral fibers. Mallory's triple connective tissue stain. × 356.

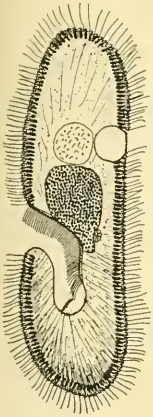
Fig. 24. Diagonal section, 2.5μ thick, through the cytostome and cytopharynx, showing cilia and "ciliary rootlets." × 1160.

Fig. 25. Cross-section, 2.5μ thick, showing cilia, "ciliary rootlets," also trichocysts and fibers running from them into the endoplasm. × 1160.

Fig. 26. Showing anterior face of cytopharyngeal fibers and peristomal fibers extending obliquely caudad into the region of the posterior membranelle zone and into the endoplasm, also the ciliary lines of part of the oral and right sides with the ciliary suture. × 356.

Fig. 27. View of right side, showing aboral trichocyst whorl and part of oral trichocyst whorl. × 357.

Fig. 28. View of aboral side, showing aboral whorl of trichocysts. × 356.



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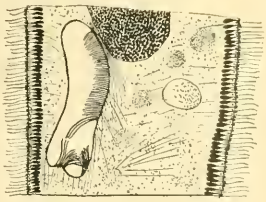

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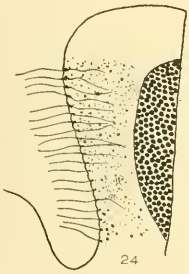

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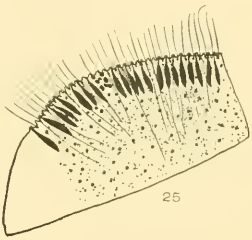

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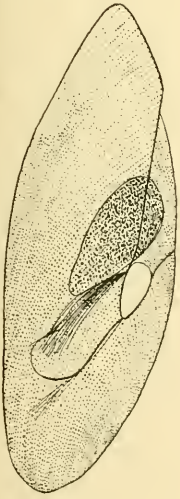

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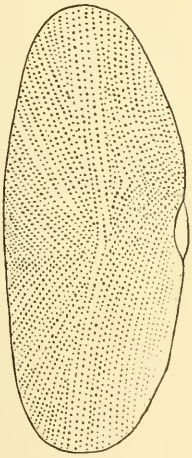

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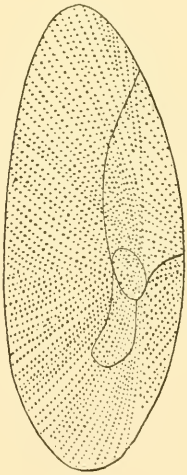


28

PLATE 36

All figures of whole mounts. × 356.

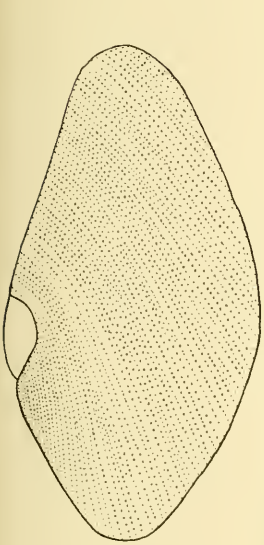
Fig. 29. View of left side, showing ciliary lines.

Fig. 30. Oral view showing ciliary lines and ciliary suture.

Fig. 31. Oral view showing oral whorl of trichocysts.

Fig. 32. Aboral view showing ciliary lines.

Fig. 33. View of left side, showing trichocysts of the oral and aboral whorls.



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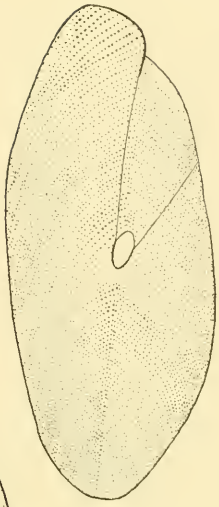

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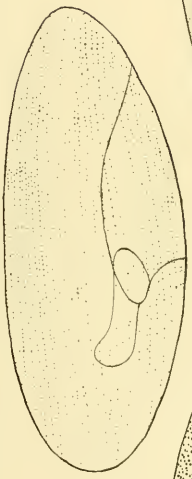

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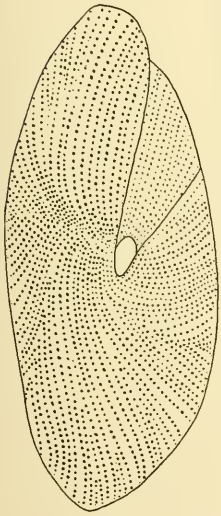
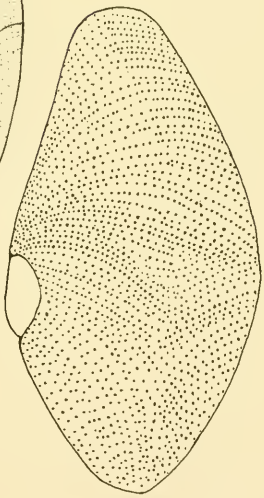


31



33

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    Nos. 17 and 18 in one cover. January, 1919 ..... .35
    Index, pp. 521-529.

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    Index in preparation.

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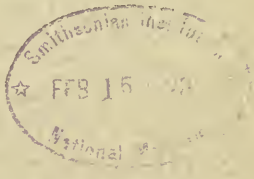
Vol. 20, No. 15, pp. 365-371 February 1, 1923

A COMPARISON OF THE CYSTS OF ENDAMOEBA COLI AND COUNCILMANIA LAFLEURI IN CONGO RED

BY
NATHANIEL BERCOVITZ, M.D.



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A COMPARISON OF THE CYSTS OF ENDAMOEBA COLI AND COUNCILMANIA LAFLEURI IN CONGO RED

BY

NATHANIEL BERCOVITZ, M.D.

Early in January, 1922, I conducted a series of experiments in the protozoological laboratory at the University of California to determine the permeability of the cysts of intestinal protozoan parasites by a number of anilin stains. During the course of the experiments it was noticed that there was no staining of the cysts by aqueous solutions of the stains until one day a number of cysts of *Councilmania lafleuri* Kofoid and Swezy were seen to appear red after being exposed to Congo red. The next specimen contained cysts of *Endamoeba coli*, and exposure to Congo red showed no reaction in the cysts which appeared clear in the red background. A third specimen contained cysts of *Councilmania* and these also took the stain when exposed to Congo red. This seemed to be unusual, so it was decided to make an intensive study of this phenomenon. At first it was thought that this might be a quick way to differentiate between these two types of amoeba, but as more cases were studied it was found that the phenomenon was not constant. There were instances where the Congo red had no effect on the cysts of *Councilmania*, and there were instances where the Congo red stained cysts of *Endamoeba coli* deeply.

In order to arrive at some more accurate conclusion a series of fifty cases was studied. Fresh solutions of Congo red, 1 per cent in normal salt, were used throughout. The effect of this stain on every type of cyst of human intestinal parasites available was studied, but in the cysts of *Councilmania* and *Endamoeba coli* only was there any

staining. Some twenty-five of the more common anilin stains in 1 per cent normal salt solution were also used on these cysts, which included those of *Endamoeba dysenteriae*, large and small races; *Endamoeba nana*, small race; *Iodamoeba buetschlii; Chilomastix davainei*, and *Giardia enterica*, in addition to *Councilmania* and *Endamoeba coli*. In no cyst, and with no stain, was there any staining of live cysts except with the Congo red on *Councilmania* and *Endamoeba coli* and, as already stated, this stain acted differentially on the whole between these two.

There were a number of variables to be considered. First of all there was the matter of determining whether the cysts were alive or dead, as the dead cysts stained intensively with any of the dyes used. It was soon found that in Congo red dead cysts stained a deep orange red with nuclei showing very dark; it also became evident that in live cysts the dye did not penetrate the cyst wall, therefore did not stain either the cytoplasm or the nuclei.

This fact was therefore established early, that in live cysts the dye did not stain the cytoplasm or nuclei of the cyst, but only the cyst wall. It was also found that there were degrees of intensity of the staining of the cyst wall, and for this reason it was decided to designate three degrees of staining as follows: the intense shade as "stained," the light shade "pinkish," and where no staining was apparent as "unstained." It was found in many of the cysts which were ordinarily regarded as "unstained," that in a powerful light a faint pinkish shade might be seen. This might have been due, however, to the film of staining fluid around the cyst.

The other variables were the amounts of material used and the dye used. Obviously both of these could not be kept absolutely constant, but continuous practice reduced to a minimum error due to these factors.

After a few trials it was found that if a dye was going to act at all on a cyst it acted immediately, and that the cyst did not stain more intensively if exposed for a long time, even as long as two days in the dye. So there was no error due to the time element. Variations in room temperature did not make any difference in the results.

It is obvious that in a study like this the personal element enters in a large way, since what would be considered stained by one might be considered pinkish by another, and even with the same person, there would be variations of judgment as to the intensity of the color, especially with different densities of the surrounding field of material

and stain. For this reason this study is not absolute, but the findings are recorded as a general result and are liable to the variations of personal and other elements.

Early in the study it was noticed that in stools in which *Councilmania* were found the cysts seemed to be more numerous than in those containing *Endamoeba coli*. This feature was also studied in connection with the action of stains.

The results of the findings in the first fifty cases are recorded in tables 1, 2, and 3. In general it was found that the cysts of *Councilmania* when present are abundant in the stool, while on the other hand those of *Endamoeba coli* are usually very scarce, at times not more than one or two cysts being found on a slide. It was also found that the tendency of the cyst wall of *Councilmania* to take the Congo red was much more marked than in the case of *Endamoeba coli*. Furthermore, the cysts of *Councilmania* seem to be ovoidal or ellipsoidal oftener than the cysts of *Endamoeba coli*.

In the first series of 50 cases there were 17 cases, or 34 per cent, with *E. coli* infection; 29 cases, or 58 per cent, with *Councilmania* infection, and 4 cases, or 8 per cent, with infection of both *E. coli* and *Councilmania*.

TABLE 1

SERIES I. RELATIVE ABUNDANCE OF CYSTS OF E. COLI AND COUNCILMANIA IN SLIDES

	Few	Moderate	Many
Endamoeba coli	64.7%	23.6%	11.8%
Councilmania	17.2%	10.4%	72.4%

This table indicates that *Councilmania* cysts are more abundant in stools than are the cysts of *Endamoeba coli*.

TABLE 2

SERIES I. REACTION OF CYSTS OF E. COLI AND COUNCILMANIA TO CONGO RED

Type of Cyst	No. of Cases	Unstained		Pinkish		Stained		Pink and Unstained in same Specimen		Total No. Showing Color	
		No.	%	No.	%	No.	%	No.	%	No.	%
E. coli	17	9	53.1	6	35.4	2	11.8	0	0	8	46.9
Councilmania	29	6	20.7	3	10.3	9	31.0	11	37.9	23	79.3

This table shows that the cyst wall of *Councilmania* takes the Congo red stain more readily than that of *E. coli*.

In order to place these conclusions on a more accurate basis, a second series of fifty stools was examined in which the following factors were noted: cysts dead or alive in stool; stool formed or liquid; actual count of cysts on slides to determine more accurately the proportion staining, as well as abundance of cysts in these cases.

Congo red itself being a very delicate indicator, it was noted whether the stool became dark in the Congo red, and this was used as the test for acidity.

Where cysts were abundant one hundred were counted, and the results with the stain noted. But in no case were more than two slides of each specimen examined, as in many cases it would have required examination of ten to fifteen slides to find one hundred cysts. Where one hundred cysts were found on one slide it was considered that the cysts were abundant. Where less than one hundred cysts were found on one slide, but more than fifty cysts on two slides, the cysts were considered to be moderately abundant. Where there were less than fifty cysts on two slides the number was considered few, and where less than ten cysts on two slides, very few.

The results of the second series are recorded in tables 3 and 4. In this series 22 cases, or 44 per cent, were infected with *Endamoeba coli*, and 28 cases, or 56 per cent, were infected with *Councilmania*. No mixed infections were included in this series.

TABLE 3

Series II. Relative Abundance of Cysts of E. coli and Councilmania Cysts

	Few (including very few)	Many (including moderately abundant)
E. coli	86.4%	13.6%
Councilmania	21.5%	78.5%

The contrast between abundance of cysts in infections by *Endamoeba coli* as compared with infections by *Councilmania* is very striking, and is of value because the cysts were actually counted. The three groups of table 1 have been changed to two large groups as being more accurate. Wherever there were less than fifty cysts on two slides it was recorded as few, and where more than fifty cysts were found on two slides the case was recorded as many.

TABLE 4

SERIES II. REACTION OF CYSTS OF ENDAMOEBA COLI AND COUNCILMANIA TO CONGO RED

Type of Cyst	No. of Cases	Unstained		Stained (showing any color at all)		Unstained				Stained			
						Many		Few		Many		Few	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E. coli	22	8	36.3	14	63.7	1	12.5	7	87.5	2	14.5	12	85
Councilmania	28	3	10.7	25	89.3	2	66.6	1	33.3	20	80.0	5	20

In this series eleven cases, or 22 per cent, showed no coloring of any of the cysts. In the first series fifteen cases, or 30 per cent, showed no coloring. In the second series the contrast between the staining in the two types is marked. The relative difference between percentage of cysts of each type stained is practically the same for the two series, the second series showing an actual higher percentage of all cysts which are stained.

In both series the diagnosis between *E. coli* and *Councilmania* was made after examination of slides stained with iron haematoxylin.

In the first series there were four cases where *Councilmania* and *E. coli* were found in the same specimen. An attempt was made to record the location of cysts which took the Congo red and of those which did not; the slides in these cases were stained in haematoxylin, in order later to find the marked cysts and thus to determine whether they were *E. coli* or *Councilmania*. Inasmuch as in these four cases both stained and unstained cysts were found, it would have been of the greatest value if it could have been determined whether all of the stained cysts were *Councilmania* and the unstained cysts *E. coli*, or vice versa. However, another very curious phenomenon was observed in these cases, in that after the Congo red had come in contact with the cyst wall the haematoxylin did not seem to have free access to the cyst; thus the cytoplasm and nuclei were not stained, and the cysts could not be differentiated.

Repeated trials of this were made, and in every case the result was the same. A large number of the cysts were distorted, and some seemed to be more or less collapsed. The haematoxylin seemed to cling to the cyst wall with great tenacity, as the cysts were exceedingly difficult to destain. An investigation of this phenomenon is to be reported later.

The results of the second series of fifty cases are in the main similar to the results recorded in the first series. The incidence of

Endamoeba coli infection is somewhat higher than in the first series of fifty, but still considerably lower than the number of *Councilmania* infections. As regards abundance of cysts, there are strikingly few cysts in the great percentage of cases infected with *Endamoeba coli*, while nearly 80 per cent of the cases infected with *Councilmania* show abundance of cysts. In cases of *E. coli* infection, a smaller number show absolutely no cysts staining at all. In many cases, however, perhaps only two or three cysts showed any color, yet these cases were classed as specimens showing color. In this second series *Councilmania* again showed a preponderance of specimens in which cysts take the Congo red stain.

TABLE 5

Series I and II. Relative Abundance of Cysts of E. coli and Councilmania in One Hundred Cases

	Cysts			
	Many and Moderate		Few	
	No.	%	No.	%
E. coli	5	14.7	34	85.3
Councilmania	46	80.7	11	19.3

Four mixed cases of first series not recorded.

In the total series of one hundred cases there were thirty-nine cases showing *E. coli* infection; fifty-seven cases showing *Councilmania* infection, and four cases with both *E. coli* and *Councilmania*.

Table 6

Action of Congo Red on E. coli and Councilmania in Ninety-six Pure Infections

	E. coli		Councilmania	
	No. of Cases	%	No. of Cases	%
Unstained	17	43.6	9	15.7
Stained	22	56.4	48	84.3

Acknowledgments are made to the California State Board of Health for the supply of material used, to the University of California for assistance from the grant made in aid of research on human intestinal protozoa, and for the use of the facilities of the protozoological laboratory, to the China Medical Board for a fellowship permitting this study, and to Professor Kofoid and Dr. Swezy for counsel.

SUMMARY

1. In infections by *Endamoeba coli* the cysts are present as a general rule in small numbers; while in infections by *Councilmania* the cysts are abundant.

2. In general the cysts of *Councilmania* take the Congo red stain whereas the cysts of *E. coli* either do not take the stain, or else in most cases take it very faintly.

3. Of all cysts of intestinal protozoa tested with some twenty-five anilin stains, only cysts of *E. coli* and *Councilmania* took any of the stains at all, and these two took only the Congo red. Furthermore, only the cyst wall was stained, the dye apparently not passing through the wall and thus not staining either cytoplasm or nuclei.

4. The cysts of the common intestinal protozoa when dead are quickly penetrated by anilin dyes and the protoplasm is deeply stained. Living cysts resist penetration by these dyes.

5. The shape of cysts of *Councilmania* in many cases is ovoidal, or ellipsoidal, whereas cysts of *Endamoeba coli* are as a rule spherical, and also slightly smaller than *Councilmania* cysts (see Kofoid and Swezy, 1921, *a*, *b*).

6. The condition of the stool, i.e., whether formed or unformed, whether acid or alkaline, had no effect either upon the abundance of the cysts or the way in which they reacted to the Congo red stain. There was no evidence that in the more fluid stools the cysts were less protected than in the formed stools.

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Transmitted, December 8, 1922.

LITERATURE CITED

Kofoid, C. A., and Swezy, O.

1921*a*. On the free, encysted, and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine. Univ. Calif. Publ. Zool., **20**, 170–189, pls. 18–22, 3 figs. in text.

1921*b*. *Councilmania lafleuri*, a new amoeba of the human intestine. Proc. Soc. Exper. Biol. Med., **18**, 310–312.

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ON THE MORPHOLOGY AND BEHAVIOR OF PENTATRICHOMONAS ARDIN DELTEILI (DERRIEU AND RAYNAUD)

BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

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ON THE MORPHOLOGY AND BEHAVIOR OF PENTATRICHOMONAS ARDIN DELTEILI (DERRIEU AND RAYNAUD)

BY
CHARLES ATWOOD KOFOID AND OLIVE SWEZY

CONTENTS

	PAGE
Introduction	373
Acknowledgments	375
Material	376
Morphology	378
Reproduction	382
Behavior	382
Survival in water	385
Culture	386
Summary	387
Literature cited	388
Explanation of plates	390

INTRODUCTION

The evolution of the polymastigote flagellates has progressed by two methods: (1) increase in the number of the anterior or other flagella, and (2) differentiation in structure and specialization in function among the flagella. The first method has given rise ultimately to the Hypermastigina which, in their most highly specialized genera, are covered over their entire surface by flagella, hundreds or thousands in number. The second method has resulted in the development within the Polymastigina of organs such as the trailing, ribbon-like flagellum of *Devescovina*, the undulating membrane and the axostyle of *Trichomonas*, and the flagellar lines of *Pyrsonympha*. The morphological constancy in number of these fundamental organelles

within each of the genera and species of the Polymastigina may be considered as established by the numerous investigations of the varied members of this widely distributed group of (mainly) parasitic protozoans.

The only period in the life of the individual when this morphological constancy in number is modified is the temporary phase of promitosis. During this phase the neuromotor organelles are doubled in number preparatory to mitosis of the nucleus and asexual reproduction by binary fission of the individual, a process which insures the morphological and numerical constancy of these organelles for the two individuals thus produced. Observed variations within the species in the number of flagella, or of other organelles formed by modification of flagella, are attributable to the onset of mitosis, and may be demonstrated to be such by an analysis of the complete mitotic process.

The importance of recognition of this deduction as a guiding principle in the study of parasitic flagellates and of its practical applications in the diagnosis of human flagellate infections as well as those of other animals is emphasized by Dobell and O'Connor's (1921) treatment of the number of flagella in *Trichomonas hominis* in their Intestinal Protozoa of Man. In this work Dobell (p. 68, footnote) states that, "In my experience the four-flagellate form (typical *Trichomonas*) is the commonest in human stools, but the 3-flagellate variety is also common. C. D." They also include the 5-flagellate *Pentatrichomonas* in the synonymy of *Trichomonas*. The reader is thus led to infer that *Trichomonas hominis* has three, four, or five flagella.

Their erroneous treatment of this matter is not only unsound morphologically, but in our judgment will lead to incorrect diagnoses of infections and will deter clinical study of, and therapeutic efforts to cure, human trichomonad infections. It tends to confuse distinct specific entities and to obscure a possible etiological factor in human disease by the as yet pure assumption that the intestinal "*Trichomonas*" in man belongs to one species only and that clinical accounts of pathogenic conditions associated with such infections are always to be explained by the further assumption of the concurrence of another unobserved etiological cause.

No review of the conflicting clinical literature on intestinal trichomoniasis will be attempted here since it has recently been made elsewhere by Haughwout (1918), Kofoid and Swezy (1921), and

Dobell and O'Connor (1921), the latter decrying but by no means establishing the innocuousness of these infections. Intensive and repeated clinical studies by competent clinicians, with the infecting organism determined by competent protozoologists, will be necessary to settle the mooted point of pathogenicity. The complex interrelations in disease and the widespread occurrence of the carrier phase in many infections capable under some conditions of inducing disease, lead one to hesitate to accept as soundly scientific Dobell and O'Connor's sweeping assumptions of non-pathogenicity or their summary dismissal, as being without significance, of the observations of others as to tissue penetration and pathogenicity. More investigation, rather than a dogmatic dismissal, is sorely needed.

In our experience the common trichomonads of the human intestine, urogenital tract, and mouth all have four anterior, free flagella. In case three only are seen at first, a fourth can be found if the material is so prepared as to be adequately examined. In our experience failure to find the fourth has been due to the belief that three is a normal number, or to inexpertness in analysis of specimens, or to the disadvantageous position of one of the four flagella, as, for example, when it is hidden under the body. Fundamentally, the reason lies in a failure to observe the differential behavior of one of the anterior flagella. It is the purpose of this paper to give an account of several infections by *Pentatrichomonas ardin delteili* (Derrieu and Raynaud) in man and to direct the attention of protozoologists to the distinctness of this genus and of clinicians to the desirability of distinguishing this organism from the seemingly innocuous and more widely prevalent *Trichomonas hominis*.

ACKNOWLEDGMENTS

We are indebted to Dr. L. M. Boyers, the attending physician on our principal case, for coöperation, and to the patients for prolonged coöperation in providing material for research. Acknowledgments are also made to the Carnegie Institution of Washington, to the Board of Research of the University of California, to Margaret B. Fowler, and to an alumnus of the University of California, for grants which have made possible this investigation, as well as others, on human intestinal Protozoa.

MATERIAL

In the course of 25,000 examinations of 7010 persons made by us up to November 30, 1922, the latest date to which our statistics have been assembled, we recognized 75 cases or 1.1 per cent infection by *Trichomonas hominis*. The actual percentage of infection was doubtless higher, since in the absence of cysts detection rests upon finding living flagellates, and these in some types of stool do not survive long and are usually infrequent in formed stools. In our experience this species, except in dividing individuals, has only four flagella.

We found three cases in which there was an infection by *Pentatrichomonas ardin delteili* (Derrieu and Raynaud). The first of these was an army officer who had resided in the Philippine Islands and Panama, and on and near the Mexican border. Nineteen examinations of this case were made over a period of nine months. In each case *Pentatrichomonas*, and this flagellate only, was detected. There was a concurrent infection by *Strongyloides stercoralis* and by *Blastocystis*. The patient had a previous history of dysentry, had received treatment for amoebiasis (emetin-bismuth-iodide and neoarsphenamine) and had gained in weight after this treatment. Chronic diarrhoea marked the attack here treated. No free amoebae and no amoebic cysts of any sort were found in any of the stools examined by us, though diligently sought.

The second case was also an army officer who had been stationed for thirteen months on the Western Front in France, for a time in an area where French African colonial troops and Chinese laborers were billeted. To date seventy-eight examinations have been made on this case. No worm infections have been found. There are concurrent infections by *Chilomastix davainei* and *Blastocystis*. *Pentatrichomonas* was not found in only thirteen of these stools. In some instances this was due to the condition of the stool resulting from delay in shipment of the specimen from a distance. *Chilomastix* was found in twenty-seven of the seventy-eight stools and was rarely abundant. No other animal parasite was found in these examinations. (Shortly before our examination began the patient had been found to have amoebiasis and had received the emetin-bismuth-iodide and arsphenamine treatment.) No amoebae, free or encysted, were at any time found by us in his stools.

The third case was a female school teacher resident for eighteen years in the Hawaiian Islands. Fifteen examinations have been made to date of this case, all positive for *Pentatrichomonas*, but no other infection, either protozoan or helminth, has been detected. There is no clinical history of amoebic dysentery in this case.

In all three cases there is a record of a chronic diarrhoea with purulent, fetid, yellowish-brown stools, rarely with mucus, with but little or no blood, and of a smooth consistency. Defecation was frequent, sometimes as many as twenty stools a day. Semi-formed or formed stools rarely occurred. The customary palliative measures failed to give relief. Microscopic examination revealed enormous numbers of *Pentatrichomonas* swarming in the fluid stool. In semi-formed stools the numbers were lessened.

These three infections, all with similar symptoms and stools, presented only *Pentatrichomonas* with five flagella, never *Trichomonas hominis* with four; at least we found only the former in every critical determination of the number of anterior flagella. We therefore conclude that *Pentatrichomonas* is distinct from *Trichomonas*.

Generic status is desirable for *Pentatrichomonas* in view of the general use of the number of flagella as the basis of generic distinction in the Polymastigina. We therefore utilize the generic name proposed by Chatterjee (1915). The species was originally described by Derrieu and Raynaud (1914) as *Hexamastix ardin delteili* from a fatal case of diarrhoea originating in Algiers but observed in Paris. It was also described at about the same time by Chatterjee (1915) as *Pentatrichomonas bengalensis* from a case of chronic diarrhoea at Calcutta. Mesnil (1915*a*, *b*) noted the similarity of the two organisms, called attention to the preoccupation of *Hexamastix* by a different flagellate described by Alexeieff (1912) and recognized the availability of *Pentatrichomonas* as the generic name. He held open the possibility of specific distinctness of the Algerian and Indian species. The nature of the similarities of the two, however, and the great variability of the species in our material make it certain, in our opinion, that the two supposed species are identical. Chatterjee (1917) later reported this species in 32 of 70 cases of flagellate diarrhoeas observed at Calcutta. Wenyon and O'Conner (1917) reported one observation of a five-flagellated trichomonad from man observed in Egypt. Haughwout (1918, 1919, 1920) reported *Pentatrichomonas* in cases of diarrhoeas at Manila and described the method by which they engulf and seemingly digest red blood cells with which they

may be associated in the stools. It appears from these records of occurrence that *Pentatrichomonas* may have a wide distribution in the tropics.

It is important that the occurrence of epidemics of trichomonad diarrhoeas reported from Colombia by Alvarez (1916), from Argentina by Vaccarezza (1917), and from Arequipa by Escomel (1919), be critically examined with reference to the presence of *Pentatrichomonas.*

MORPHOLOGY

Pentatrichomonas ardin delteili has the typical organelles of the trichomonad flagellates, namely, (1) the neuromotor system consisting of the anterior flagella, undulating membrane, axostyle, blepharoplasts, centrosome, and rhizoplasts, and (2) the other non-fibrillar organs, the nucleus, and the cytostome. These differ structurally, if at all, only in minor details of proportions from those of other species of trichomonads.

The distinguishing characteristic of the genus is the presence of *five* anterior flagella. The genus *Trichomonas* Donné (1837) has four and *Tritrichomonas* Kofoid (1921) has three.

These flagella are slender threads of uniform and equal caliber whose length exceeds slightly that of the body in normal extension.

These anterior flagella fall into two groups as to origin and behavior, a fact not hitherto noted and ignorance of which tends to obscure the number of flagella. These may be designated respectively as the single independent (*ind. ant. flag.*) and the three clustered anterior flagella (*clust. ant. flag.*, fig. A).

The two groups arise from different blepharoplasts. The independent flagellum arises, together with the undulating membrane, the parabasal body, and possibly the axostyle also, from the proximal or primary blepharoplast (*prim. bleph.*, fig. A), while the three clustered anterior flagella arise from the more distal or secondary blepharoplast (*sec. bleph.*, fig. A).

Dobell and O'Connor (1921) state that there are at least three and possibly more blepharoplasts and that the flagella appear to take their origin from at least two of these; but they do not distinguish the two groups of flagella nor state their relations to the blepharoplasts, nor that of the other organelles to these structures. Their figures (their pl. 5, fig. 71) might be interpreted as showing two

blepharoplasts and a centrosome. It is possible that the granule at the base of the clustered flagella may be subdivided, or that there may be a granule at the tip of the axostyle, but we have been unable to find clear evidence of more than two blepharoplasts with the relations as above defined by us.

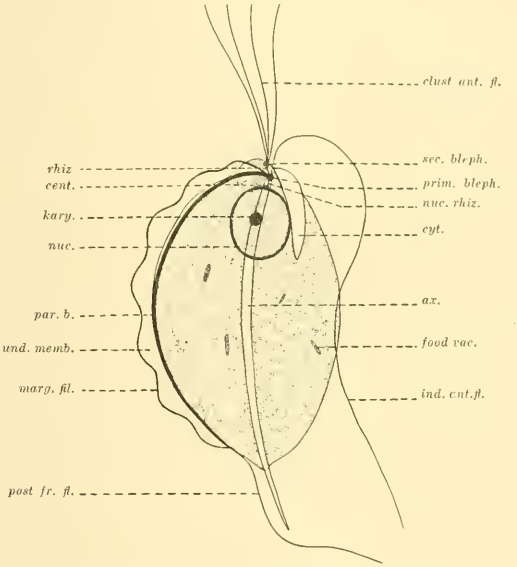


Fig. A. *Pentatrichomonas ardin delteili.* × 3200. From stained preparation of human stool. Abbreviations: *ax.*, axostyle; *cent.*, centrosome; *clust. ant. fl.*, clustered anterior flagella; *cyt.*, cytostome; *food vac.*, food vacuole; *ind. ant. fl.*, independent anterior flagellum; *marg. fil.*, marginal filament: *kary.*, karyosome: *nuc.*, nucleus; *nuc. rhiz.*, nuclear rhizoplast; *par. b.*, parabasal body; *post. fr. fl.*, posterior free flagellum; *prim. bleph.*, primary blepharoplast; *rhiz.*, rhizoplast; *sec. bleph.*, secondary blepharoplast; *und. memb.*, undulating membrane.

The two blepharoplasts are joined by a very short rhizoplast and the proximal one is connected with the centrosome (*cent.*, fig. A) on the nuclear membrane by a definite rhizoplast (*rhiz.*, fig. A).

The centrosome (*cent.*, fig. A; pl. 37, figs. 1, 2, 7) is a small, deeply staining granule on the anterior surface of the nuclear membrane in close juxtaposition to the blepharoplasts. At certain stages of mitosis an intranuclear rhizoplast (pl. 37, fig. 10) may be seen to

connect the centrosome with the central karyosome as it sometimes does in other flagellates, such as *Giardia* (Kofoid and Swezy, 1922).

The undulating membrane (*und. memb.*, fig. A) extends from the primary blepharoplast posteriorly in a slightly leiotropic spiral (pl. 37, fig. 4) of not over 0.5 turn at the most and extending posteriorly about 0.8 of the length of the body to the end of the parabasal body, where it terminates with the emergence of the marginal filament as the posterior free flagellum. The number of undulations present in this membrane at any one time appears to be a function of their rate of progress. They are not always evident in fixed material. In active free flagellates they move so rapidly as to defy estimation. In fixed material one finds at the most (pl. 37, figs. 1, 7) eight to ten undulations.

The membrane consists of the marginal filament (*marg. fil.*, fig. A) which is morphologically a flagellum originating from the primary blepharoplast, enclosed within the outer pellicle, and terminating distally in a free flagellum (*post. fr. flag.*, fig. A) which is about 0.5 of the length of the body or more. It also contains a ribbon-like extension of the periphery of the body at whose base runs the parabasal body (*par. b.*, fig. A). This structure is a curved rod of uniform caliber and regular course, originating from the primary blepharoplast and following the course of the undulating membrane. It terminates at the point of emergence of the posterior free flagellum. It stains lightly and destains very readily in our material and is often located with difficulty, if at all.

The axostyle (*ax.*, fig. A) is a stout rod of hyaline appearance and uniform caliber, which appears to originate from the primary blepharoplast and to extend posteriorly in an axial position. Its posterior end projects beyond the cytoplasm as a rather bluntly pointed tip for varying distances, depending upon the amount of cytoplasm present and upon the degree of contraction of the body. Its free end rarely attains a length of 0.5 that of the cytoplasmic body. Its varying degree of curvature in different individuals is suggestive of the contractile function which we have seen and described in *Trichomonas* (1915).

The cytostome (*cyt.*, fig. A) is a well-defined and characteristic structure. It is an elongated, broadly comma-shaped, often slightly protuberant area (pl. 37, figs. 3, 6) on the opposite side of the body from the undulating membrane. It extends from the anterior tip of the body posteriorly for about 0.35 of the length of the body and

tapers posteriorly asymmetrically. A dark fibrillar line forms its margin. We are unable to find a fibrillar connection between this margin and a blepharoplast such as we found (1920) in *Chilomastix*. It abuts directly upon the secondary blepharoplast and may have a connection with it.

The nucleus (*nuc.*, fig. A) varies greatly in size, ranging from two to four microns in diameter. It is smaller directly after mitosis in some instances (pl. 37, fig. 5). It is usually spheroidal, but occasionally ellipsoidal with the longer axis anteroposterior. It is located anteriorly, never more than 0.5 its diameter from the blepharoplasts and usually almost in contact with them. Its membrane is lightly encrusted with peripheral chromatin and at its center there is often a single, spherical karyosome of variable size (pl. 37, figs. 3, 4). In some instances there are two such chromatin masses (pl. 37, figs. 2, 7) which suggest the early prophase of mitosis. In one instance (pl. 37, fig. 10) of a late telophase, an intranuclear rhizoplast runs from the centrosome to the chromatin mass.

The cytoplasm of this species is generally rather coarsely vacuolated, except during binary fission. The vacuoles may contain red blood corpuscles in various stages of digestion, or clusters of bacteria or food remnants (pl. 37, fig. 3). We have not observed in this species the sloughing off of cytoplasmic blobs which we have previously noted in *Trichomonas* (1915) and *Chilomastix* (1920).

The shape of the body on stained slides is stout pyriform, tapering posteriorly with a slight asymmetrical taper anteriorly also to the region of the blepharoplasts. It is more rotund on the side of the cytostome. Posteriorly it tapers in an asymmetrical cone of forty-five or more degrees. The taper at the two ends is such as almost to give an asymmetrical biconical shape to the body, the convexity is somewhat greater on the side of the undulating membrane. In life the length is often twice the greatest diameter, or even more, and the asymmetry of the apex more marked than in stained individuals.

The dimensions of *Pentatrichomonas* in fresh stools and in cultures vary considerably. The length of twenty-five individuals measured from the anterior tip of the body to the posterior end of the cytoplasm, but not to the tip of the axostyle, ranged from 9 to 20 microns, averaged 14.6 and had its mode at 14 microns. The width ranged from 7 to 14 microns, average 9.3 and had its mode at 10 microns. A rounded-up individual measured 9 by 9 microns. These measurements were made on material stained in iron haematoxylin. In life

the length is as a rule somewhat greater, contraction undoubtedly occurring as individuals slow down, and in fixation. The length of the exposed tip of the axostyle was 2 to 4 microns. The diameter of the nucleus ranged from 2 to 4 microns. The transdiameter in the plane of the undulating membrane was greater by about one-tenth than that at right angles. The optical cross-section was broadly pyriform with the narrower end at the undulating membrane.

REPRODUCTION

Binary fission (pl. 37, figs. 8–10) preceded by mitosis occurs abundantly in stools and in cultures. We have repeatedly seen multiple fission or somatella formation in cultures. A two-, four-, and eight-nucleate somatella of spheroidal shape only slightly larger than the unicellular phase with a neuromotor system attached to each nucleus is formed. This presumably undergoes plasmotomy and forms eight unicellular flagellates. We have never seen the least trace of encystment in either fresh stools or cultures. Efforts to induce encystment by slow desiccation of stools and cultures were also fruitless.

BEHAVIOR

Observations on living *Pentatrichomonas* in liquid stools and especially in the marginal zone between normal saline and iodine-eosin stain, where movement is progressively slowed down, has enabled us to analyze the movements of certain elements of the neuromotor system and to trace certain stereotyped features of the behavior of the animal, and especially to determine the differential movements of the single independent, and the clustered anterior, flagella.

Locomotion in *Pentatrichomonas* is continuous in normal individuals. It is accompanied by an anticlockwise rotation, which at times appears to be interrupted by a very brief interval of a clockwise direction. Progression may be in a straight line for short distances, but is generally curved and frequently interrupted by brief intervals of circling movement in short arcs of shorter radius in an anticlockwise direction.

A quick spasmodic contraction precedes these interruptions in speed and direction. This appears to be analogous to the motor or

avoiding reaction of the ciliates. It can be observed most readily in case of an individual temporarily anchored by the tip of the axostyle or by the free flagellum.

A peculiar, rather regular wobbling characterizes the locomotion, which appears to be due to a pendulation of the body about the axis of progression which is correlated with the differential strokes of the independent and clustered anterior flagella. As a result, the two ends describe approximately equal cones of rotation about the spiral axis of progression.

A persistent study of the location of the flagella in stained preparations and during the slowing-down period in the margin of the iodine-eosin stain in fresh smears has enabled us to distinguish the differential strokes and to correlate them with the movement of the undulating membrane and with the origin of the two groups from the two blepharoplasts.

The motion of the undulating membrane, as the slowing-down process ensues, is resolved into a series of regular, successive beats or contractions, which traverse the membrane as a series of characteristic undulations. These do not, however, appear to run through the free flagellum at the end. Each beat is synchronous with the stroke of the independent anterior flagellum. This flagellum moves independently of the other four, in a sweeping continuous stroke, starting from the anterior position, across the right face of the body, assuming that the cytostome defines the ventral side. This flagellum and the undulating membrane arise from the same primary blepharoplast and beat synchronously.

The four remaining clustered anterior flagella all beat in a stroke independently of the one just described. They appear to beat together or in rapid and close succession so that in fixed material they are often found in juxtaposition or closely spaced. They all arise from the secondary blepharoplast.

Not only are the strokes of the derivatives of the blepharoplasts independent but they beat at different intervals. In one individual in the early phases of slowing down, the strokes were carefully timed. The undulations ran at the rate of 30 beats in 10 seconds. During this time the body was rotating at the rate of 3.5 rotations in 10 seconds. In another instance the rate was 10 beats in 10 seconds. *The independent anterior flagellum beats in the same rhythm.* The four clustered flagella of this last individual were beating at the rate of only 4 strokes in 10 seconds. No protoplasmic lobe was in evidence.

The four flagella stroke from the base with the tip lagging continuously through the down and up phase, stopping for an instant in an anterior cluster at the end of the stroke. They pass over the region of the undulating membrane during the stroke. The first effect is a short jerk of the body posteriorly as the stroke begins, followed by a spiral anticlockwise turn of the body in a short arc and a forward thrust.

It is obvious that only once in its circuit will the independent flagellum pass the clustered flagella. It follows that during the most of the time it is at some distance from the other and if fixed suddenly would, as a rule, lie apart from the four flagella. This we find is its usual position on our stained slides. A knowledge of this fact is of assistance in determining the number of flagella. Failure to observe it may lead to overlooking the fifth flagellum.

Another characteristic of this and of other trichomonad flagellates, often seen in moribund or slowed-down individuals, is the formation of a stumpy or finger-like protoplasmic protrusion originating near the anterior end of the body and progressing posteriorly beyond the middle in diminishing prominence. The organism described by Castellani (1905) as *Entamoeba undulans* is doubtless based on this phenomenon in a moribund trichomonad flagellate.

The exact nature of this process has not hitherto been determined. Dobell and O'Connor (1921) described it as occurring after "the organism loses its flagella and other organs." As we have observed it, it certainly may occur when all the organelles are present and functioning, though slowly. It is highly probable that it is the manifestation in slowing-down individuals of the spasmodic contraction or motor reaction of normal locomotion, but it takes place so quickly that it escapes observation as such in the rapidly moving organism. Since it varies greatly in extent when it can be observed in operation, it is quite possible that it is exaggerated in extent in moribund individuals. It is not a pseudopodium either morphologically or functionally.

Its form is that of a blunt process with rounded tip and spreading base. Its height varies but is greatest near the middle of the body or just anterior to this level. It may attain a height equal to one-half the transdiameter. In other cases the lobe is so broad at the base that it gives a subtriangular outline to the body.

The axostyle within the cytoplasm is so difficult to detect in life that one cannot determine whether or not it shares in this movement.

The probability that it is concerned both with the motor reaction and with the protoplasmic lobe should be borne in mind in the study of these phenomena.

This lobe is not formed in, nor is it a phase of, the undulating membrane. We have repeatedly watched it in individuals in which the undulating membrane was still functioning. It is formed on the side opposite to that membrane and its rhythm is much slower than that of the membrane. In one observed instance the undulating membrane had a rhythm of 30 beats in 10 seconds while the lobe had one of but 6 beats in the same time.

SURVIVAL IN WATER

This parasitic flagellate may survive for some time in liquid stools at room temperatures. The period of survival in different stools of the same persons is not the same, possibly because of varying bacterial growths. The flagellates often disappear in 3 to 5 days, but in one instance a liquid stool kept at room temperature, 65°–70°, retained living *Pentatrichomonas* from October 4 till October 27, a period of 24 days. Sterilized tap water, rain water, and creek water are less favorable for the survival of these flagellates, though not immediately destructive. The flagellates disappear in these fluids within three days following inoculation with fresh stools. This period is sufficient as to length of time for dispersal to and infection of other hosts and may afford a basis for epidemics in cases of infected water supplies.

In normal saline, three cultures survived for 8, 10, and 15 days respectively. On two vaselined slides at laboratory temperatures, flagellates in a stool diluted with normal saline multiplied and remained active for 5 to 10 days and died out in 13 and 25 days respectively.

CULTURE

Pentatrichomonas may be cultivated with facility in 10 per cent serum of the rabbit or guinea pig in Locke's solution at room temperature. Seven cultures started on September 29 in guinea pig serum ran until October 28 with a great abundance of flagellates. One culture was used for preparations on that date. One had died out by November 7, two more by the thirteenth, one by the twentieth, two by the twenty-fifth, and the last one lingered till January 24, a period of 118 days. The flagellates survived in the original stool only six days. A culture in 10 per cent rabbit serum in Locke's solution started on October 4, subcultured on October 27 and on November 9, survived without later subcultures till February 14, 98 days after the last subculture.

Using stools from this case Mrs. E. H. Wagener has cultured this organism successfully in 10 per cent human serum from Wassermann samples at 37°5 C, at present writing for 134 days, with 40 transplants at intervals of approximately 3 days. They thrive best in neutral serum (PH 7.4) and die out at 6.2 and 8.2 respectively. Of late these cultures are growing in a 'pure mixed' culture with *B. coli.*

Dobell and O'Connor (1921) discredit Escomel's (1913) statement that he had cultivated *Trichomonas hominis* by inferring, without the least evidence, that this investigator mistook free-living flagellates in his cultures for *Trichomonas.* They also discredit Lynch's (1915) successful culture of *Trichomonas* in acid broth, with the statement, "Lynch claims" and proceed to dogmatize as follows: "At the present time, however, it is not possible to cultivate this organism with certainty in any medium. All attempts which we ourselves have made have been failures." Fortunately ex-cathedra statements and negative results are easily evaluated.

We have cultivated *Trichomonas hominis* in fecal suspensions by Boyd's method and in 10 per cent guinea pig serum in normal saline, with transplants at intervals of 3 to 5 days, for several months.

SUMMARY

Pentatrichomonas ardin delteili is a trichomonad flagellate of man distinct from *Trichomonas hominis*. It has five anterior flagella while *T. hominis* has but four. The number of these flagella is constant, except as doubled in the premitotic phase, and is characteristic in the two genera. The anterior flagella fall into two groups arising from separate blepharoplasts. Four clustered flagella arise from the secondary or distal blepharoplast. The remaining single flagellum arises from the primary or proximal blepharoplast, together with the axial filament of the undulating membrane, the parabasal body, and the axostyle. The two blepharoplasts are united by a rhizoplast which continues to the centrosome on the anterior face of the nuclear membrane.

These two groups of motor organelles move in independent rhythms. The independent flagellum and undulating membrane of the proximal group run a more rapid rhythm, beating in one case of a slowed-up individual 10 strokes every 10 seconds, while the four clustered flagella of the distal group beat only 4 strokes to 10 seconds. Only once in the stroke of the independent flagellum does it lie contiguous to the four clustered ones. This fact tends to cause obscurity as to the number of flagella in this and in other trichomonads. A knowledge of it will assist in accurate diagnosis of trichomonad infections.

Pentatrichomonas eats red blood corpuscles in stools and in cultures. It persists for 5 days in the stool in the laboratory, for 3 days in water, for 15 days in normal saline, for 118 days in 10 per cent rabbit serum in Locke's solution. It can be continuously cultivated in 10 per cent rabbit, guinea pig, and human serum in Locke's solution at room and body temperatures with transplants at intervals of 3 days. Its optimum PH is 7.4.

It has been observed by us in three human cases, each with tropical residence or contacts, and each with similar symptoms of continuous, chronic, persistent diarrhoea resistant to treatment. To determine the pathogenicity of human intestinal infections, clinical study and accompanying accurate determination of the infecting species are required.

Zoological Laboratory,
University of California.
Transmitted July 9, 1923.

LITERATURE CITED

Alexeieff, A.

1912. Sur quelques protistes parasites intestinaux d'une Tortue de Ceylon (*Nicoria trijuga*). Zool. Anz., **40**, 98–105, 3 figs. in text.

Alvarez, A. V.

1916. Trichomonosis intestinal. Tesis, Bogota, pp. x + 99, 3 pls.

Castellani, A.

1905. Observations on some Protozoa found in human faeces. Centralbl. f. Bakt. Parasit. u. Inf., I. Abth., **38**, 66–69, 5 figs. in text.

Chatterjee, G. C.

1915. On a five flagellate *Trichomonas* (n. sp.) parasitic in man. Indian Med. Gaz., **50**, 1–9, 1 pl.

1917. Note on flagellate dysentery. Indian Jour. Med. Res., **4**, 393–401, pls. 25–29.

Derrieu, G., and Raynaud, M.

1914. Dysenterie chronique à flagellé nouveau. Bull. Soc. Path. Exot., **7**, 571–574.

Dobell, C., and O'Connor, F. W.

1921. The intestinal Protozoa of man (London, John Bale Sons and Danielsson), pp. ix + 211, 8 pls., 2 figs. in text.

Donné, A.

1837. Recherches microscopiques sur la nature des mucus et la matière des divers écoulemens des organes genito-urinaires chez l'homme et chez la femme (Paris, l'Auteur), pp. iv + 71, 1 pl.

Escomel, E.

1913. Sur la dysenterie à *Trichomonas* à Arequipa (Pérou). Bull. Soc. Path. Exot., **6**, 120–122.

1919. La Tricomonosis intestinal (Lima, Sammarti), 78 pp., 3 pls.

Haughwout, F. G.

1918. The tissue invasive powers of the flagellated and ciliated Protozoa with especial reference to *Trichomonas intestinalis*. A critical review. Philippine Jour. Sci., (B), **13**, 217–259, 1 fig. in text.

1920. Some current problems in protozoal dysentery. China Med. Jour., **1920**, 1–15.

Haughwout, F. G., and De Leon, W.

1919. On the ingestion of erythrocytes by *Pentatrichomonas* sp. found in a case of dysentery. Philippine Jour. Sci., (B), **14**, 207–218, 1 pl.

Kofoid, C. A.

1920. A critical review of the nomenclature of human intestinal flagellates, *Cercomonas*, *Chilomastix*, *Trichomonas*, *Tetratrichomonas*, and *Giardia*. Univ. Calif. Publ. Zool., **20**, 145–168, 9 figs. in text.

Kofoid, C. A., and Swezy, O.

1915. Mitosis and multiple fission in trichomonad flagellates. Proc. Am. Acad. Arts and Sci., **51**, 289–378, pls. 1–8, 7 figs. in text.

1920. On the morphology and mitosis of *Chilomastix mesnili* (Wenyon). A common flagellate of the human intestine. Univ. Calif. Publ. Zool., **20**, 117–144, pls. 15–17, 2 figs. in text.

1921. The flagellate infections of the human digestive tract. Nelson's Loose Leaf Encyclopedia of Living Medicine, 4, 365–398, 6 pls.

1922. Mitosis and fission in the active and encysted phases of *Giardia enterica* (Grassi) of man, with a discussion of the method of origin of bilateral symmetry in the polymastigote flagellates. Univ. Calif. Publ. Zool., 20, 199–234, pls. 23–26, 11 figs. in text.

Lynch, K. M.

1915. Trichomoniasis of the vagina and the mouth. Cultivation of the causative organism and experimental infection. (A preliminary communication.) Am. Jour. Trop. Dis. and Prev. Med., 2, 627–634.

Mesnil, F.

1914. Review of Derrieu and Raynaud's "Dysenterie chronique à flagellé nouveau" *in* Bull. Soc. Path. Exot., 7, 571. Bull. Inst. Pasteur, 12, 968.

Mesnil, F.

1915. A propos du flagellé nouveau décrit par MM. Derrieu at Raynaud. Bull. Soc. Path. Exot., 8, 574–575.

Vaccarezza, R. F.

1917. Tricomonosis intestinal. Anales Inst. Modelo Clin. Med. (Buenos Aires), 2, 1–37, 15 figs. in text.

Wenyon, C. M., and O'Connor, F. W.

1917. Human intestinal Protozoa in the Near East (London, John Bale, Sons and Danielsson), 218 pp., 4 pls.

EXPLANATION OF PLATE

PLATE 37

All figures of *Pentatrichomonas ardin delteili* (Derrieu and Reynaud) × 2500. Material fixed in hot Schaudinn's fluid and stained in iron haematoxylin. Drawings made with Abbe camera lucida.

Fig. 1. Individual with undulating membrane in lateral position, cytostome below with chromatin diffused throughout the nucleus. Note detached position of the anterior independent flagellum.

Fig. 2. 'Ventral' view with cytostome uppermost and undulating membrane below. Note curvature of axostyle, centrosome on the nuclear membrane, and two karyosomes, probably preparatory to mitosis.

Fig. 3. Lateral view with very large nucleus, large karyosome, and food vacuole with contents.

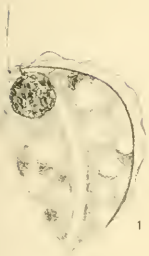
Fig. 4. Lateral view with remnants of food particles in the cytostome and chromatin encrusted nuclear membrane.

Fig. 5. Individual which had recently ingested a red blood corpuscle. From life.

Fig. 6. Lateral view of contracted specimen with much curved axostyle, and diffusely stained nucleus.

Fig. 7. Lateral view with protuberant cytostome. Note centrosome on the anterior face of the nuclear membrane, two karyosomes in the nucleus, and the straight axostyle.

Figs. 8, 9, and 10. Binary fission with two nuclei and a common cytostome, each with its independent neuromotor system.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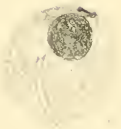
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THE PSEUDOPODIAL METHOD OF FEEDING BY TRICHONYMPHID FLAGELLATES PARASITIC IN WOOD-EATING TERMITES

BY

OLIVE SWEZY

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BY
OLIVE SWEZY

In the food-taking habits of the Protozoa we find as great a diversity as is found in other features of their life history or morphology. In those groups having a holozoic type of feeding, two general methods of taking food particles into the body may be distinguished. In the forms showing a definite polarity food is taken in at or near the anterior or head end, in common with the method general in the Metazoa. When a distinct pellicle or a structurally differentiated ectoplasm is present, as in the flagellates and ciliates, an oral aperture or cytostome is generally found, at least in those forms which ingest solid food particles.

In the amoeboid type of protozoan, with little or no evidences of polarity, food may be taken in at any point in the body. This method of feeding is the common one among the Rhizopoda, the former one being typical of the Ciliata and Flagellata.

In the remarkable and highly complex group of flagellates inhabiting the digestive tract of the termites, at least two genera of flagellates, *Trichonympha* and *Leidyopsis*, are found which combine the type of feeding of the Rhizopoda with the highly differentiated ectoplasm characteristic of the higher Ciliata. This fact, combined with the very striking polarity exhibited by these flagellates, distinguishes this group from most, if not all other Protozoa in its food habits.

In the first published description of the trichonymphids by Leidy, in 1881, mention is made of the great number of solid food particles

found within the body, and the total lack of evidence of the manner of ingesting them. Later investigators have been equally at a loss to account for their presence with the lack of a visible cytostome. Kent (1884), however, decided that an oral aperture was present at the base of the cone-like anterior portion of the body, opening into a narrow oesophageal tract which extended to the digestive cavity at the posterior portion of the body.

Porter (1897) was unable to confirm Kent's observations and offered another solution of the problem, but with little evidence to support it. He found cross-sections of the body of *Trichonympha* which showed deep folds of the body wall, invariably containing cilia in which wood particles were often entangled. He says that

> most of the cilia are probably afterwards withdrawn from the fold but the lips of the fold become so closely applied to each other that the ligneous particles are left behind in the depth of the fold. I believe that the lips afterwards fuse together, that the walls of the infolded portion then disappear, and that the food thus finally becomes entirely enclosed in the body protoplasm of the parasite.

Other investigators have not been more successful in solving the mystery of food-taking in the trichonymphids, nor has our own earlier work (Kofoid and Swezy, 1919) on these flagellates solved the problem. This has been due probably to the extreme delicacy of these organisms and the brief duration of their activities under the microscope in ordinary fluids, such as physiological salt solution. We have, however, been more fortunate in the study of the trichonymphids from a colony of *Termopsis angusticollis* (?) from Santa Cruz, California.

These termites had been living in the laboratory in a block of wood in a stone crock closely covered since January, 1920. In spite of the fact that no moisture had been added to the crock during that time and no provision made for ventilation, the termites were found in vigorous condition when removed for examination.

For study of the living organisms the largest termites were selected, those in which the abdomen seemed to be somewhat swollen. The entire intestinal tract was removed and placed upon a slide which had been ringed with vaseline. The wall of the intestine was carefully opened and spread apart and a coverslip placed over it. Usually in the largest individuals there was sufficient liquid in the intestine to make the addition of physiological salt solution unnecessary, and best results were obtained in such cases. *Trichonympha* has been kept alive and in active motion in slides prepared in this manner for two

to six hours. In physiological salt solution and various other media that have been tried, the body of the flagellate tends to round up immediately, becoming greatly distended and dissolving a few minutes after being placed on the slide.

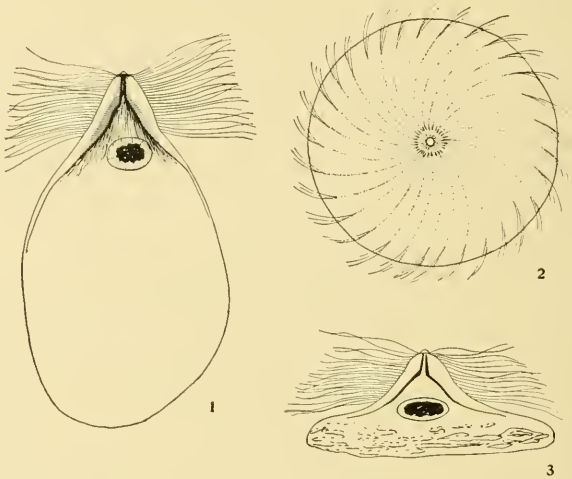
These termites contained a species of *Trichonympha* and one of *Leidyopsis* in great abundance. Both were found to be actively feeding and full details of the process could be watched under the microscope.

A slide prepared in the manner described above presents a remarkable picture of ceaseless activity and turmoil when viewed under a 1/6 objective with a low ocular. So narrow is the space between the bodies of the flagellates that often flagellar activity is entirely obscured and the resulting picture shows only a tangled mass of writhing protoplasmic bodies, the movements and appearance of which defy description. Near the outer borders of such masses the number of individuals becomes somewhat lessened and here their movements may be followed with greater ease and certainty.

The outstanding feature in such a picture is the extreme mobility of the flagellate's body. When worming its way through a crowded mass of its fellows, the change of outline is incessant, with such changes reflected in the shape of the nucleus and protoplasmic contents of the posterior part of the body. The endoplasm in the anterior end seems to partake less of this movement, though this may be due to the fact that it is very finely granular and contains no particles that would catch the eye by their movement. The anterior portion of the body, or that covered by the differentiated ectoplasm (Kofoid and Swezy, 1919*a*), is in constant motion, waves passing spirally in an antero-posterior direction. These movements are irregular, sometimes being of great frequency and amplitude, particularly when an obstacle is met, at other times becoming slower, with only a slight rise and fall of the surface. The posterior portion is usually more passive and changes its shape only to meet the requirements of its environment. When adverse conditions on the slide have slowed down the motion of the flagellate, the protoplasmic movements cease entirely, but the flagella continue their rippling motion for some time.

Leidyopsis shows the same types of movements described for *Trichonympha*, but the area of differentiated ectoplasm being much smaller, both relatively and actually, its mode of progression is much less striking than that of the larger flagellate. It exhibits two distinct shapes of body. The first (fig. 1) is elongate with the posterior

portion somewhat tapering or narrowly rounded. The second (fig. 3) is that of a shallow bell which, viewed from the anterior end (fig. 2), presents a circular or nearly circular outline. Its movements in this stage remind one strongly of those of a jellyfish, depending not so much on its flagella as on its complicated system of myonemes (Kofoid and Swezy, 1919*b*) by means of which the surface is kept in constant vibration. When in this condition its movements do not result in



Figs. 1–3. *Leidyopsis* sp. 1. Figure showing typical form. The part of the body posterior to the line showing width of ectoplasm is covered only by thin film of ectoplasm. × 400. 2. Bell-shaped individual looked at from the anterior end. 3. Same individual viewed from the side. Figures 2 and 3 were drawn from living specimens. × 300.

much change of position. The edges of the bell do not remain passive but push out in little exploratory movements.

If a food particle comes in contact with the lower surface of the bell it may be thrown off by the activities of the body, or it may be caught by the mobile ectoplasm of that region, which seems to become somewhat sticky. In the latter case the flagellate proceeds to engulf the object, even if it is of considerable size. More frequently the mobile edges of the bell may become attached to a food particle and initiate the engulfing process.

The first point of contact between the flagellate and the food body, usually a particle of wood, may be very small (fig. 4), but it is

held quite firmly, a necessary requirement in the turmoil going on around it if the animal is to feed at all. This appears to be done by means of secretions of the ectoplasm at the point of contact. A small pseudopod of clear ectoplasm may now be seen moving along the

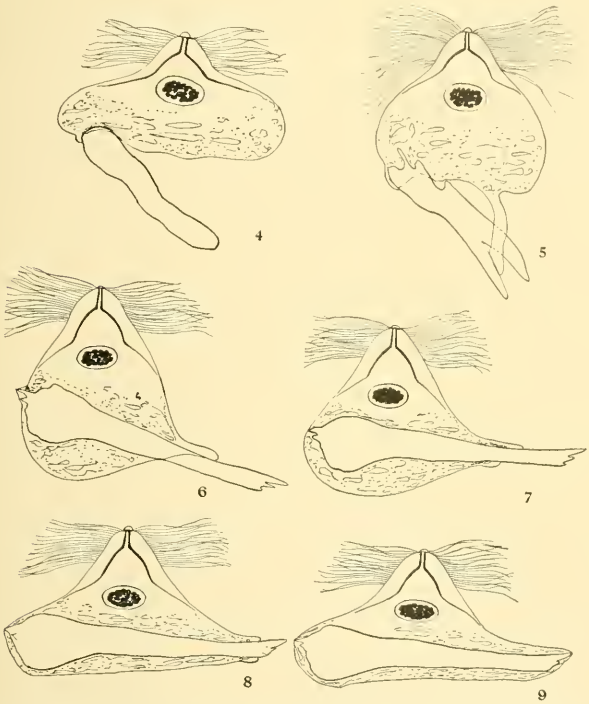


Fig. 4–9. *Leidyopsis* sp., drawn free-hand from living specimens. Figure 4 shows beginning of process of ingesting food body. Figure 5 shows a somewhat later stage. Note long clear pseudopod. Figures 6 to 9 give successive stages in the ingesting of a food body, process occupying about one hour. Note clear pseudopods creeping along wood particles. × 300.

surface of the wood (fig. 4). The pseudopod may continue along one side at first (fig. 6), or the ectoplasm may move out in a ring around the particle, drawing it into the body by one end, dependent apparently on the location of the point of its first contact. When this point is at the side the pseudopods may extend in either direction

along the length of the wood. When the protoplasmic body comes in contact with the end, that is completely engulfed first.

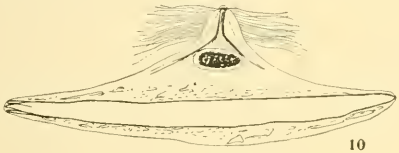
The length of time necessary for the complete intake of any particle could not be learned with exactness, owing to the tendency of the strong light used as illumination for the microscope to slow up protoplasmic movements other than those used in locomotion. In the individual shown in figures 4 to 6, the time of completing the process was found to be one hour. This individual was not held in the field continuously but was returned at short intervals, with only partial light in the interims. When first brought into the light the pseudopods could be seen moving forward with slow creeping motion. In figure 5, a flagellate is shown with one long pseudopod extending out to a branch of the woody particle that is being engulfed. This pseudopod was formed when that branch touched the surface of the body, contact being made at only one point but that strong enough to draw out the strand of clear ectoplasm when the surrounding obstacles forced the piece of wood farther away from the body (see also fig. 13).

The protoplasm shows remarkable elasticity as well as tenacity. One flagellate was seen with a piece of woody material about its own size attached by a pseudopod 10μ in length. In swimming it met obstacles which its own mobile body easily passed, but the unyielding wood was caught and the pseudopod was drawn out to about five times its original length. After great struggles the wood passed the obstacles and in a few seconds the pseudopod shortened to less than its original length, drawing the wood with it.

The body may become almost completely filled with woody particles (fig. 11), since the act of engulfing one piece does not prohibit the attempt to capture a second one at the same time by another part of the body. Only rarely is a flagellate seen without many food bodies within the cytoplasm. These seem to lie in close contact with the endoplasm in most cases but occasionally may be enclosed by a well-marked food vacuole. Many of the particles within the body of the flagellate show sharply defined outlines, while others, by their vague, structureless appearance, give evidence of the progression of digestive operations.

No evidences of selection have been found in the feeding of these flagellates. The host termite is a wood-eating species and the intestinal contents consist almost entirely of woody particles. These may be of any size from minute particles to others several times the

length of the flagellates. The larger pieces are engulfed as freely as smaller ones. Occasionally *Leidyopsis* may be found to have mastered a piece of wood so long that the body of the flagellate becomes stretched out several times its own usual width, forming a grotesque caricature of its customary appearance (fig. 10). It is also not uncommon to see pieces so long that the ends project beyond the two sides of the body.



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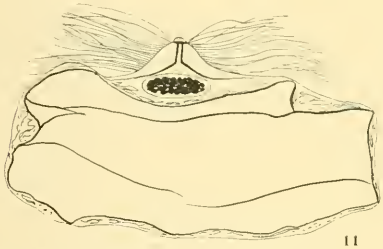

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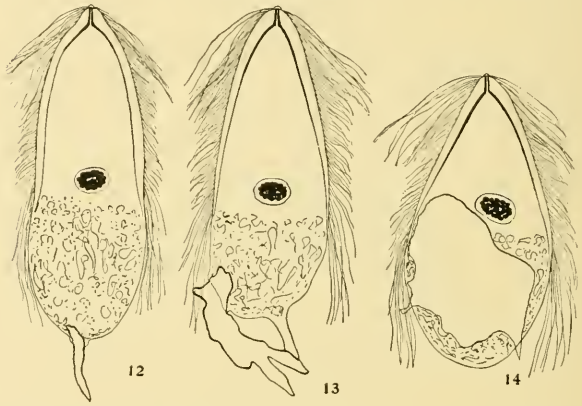
Figs. 10–11. *Leidyopsis* sp., drawn from living specimens. Note huge size of food bodies ingested. × 300.

The smaller flagellates, such as *Trichomitus*, may become the prey of both *Trichonympha* and *Leidyopsis*. An individual of the latter genus was noted with an engulfed *Trichomitus* that was rapidly rotating in a vacuole in the posterior part of the body. This individual was watched for about one hour when its movements had become slowed down. The vacuole did not have the appearance of the ordinary food vacuole but seemed to have been formed by the activities of the *Trichomitus*.

Particles may also be ejected from the body in the posterior region. The ectoplasm here lacks the structural differentiation characteristic of the anterior portion of the body as well as the ciliary coating of flagella (fig. 1). No evidences of an attempt to engulf or eject food particles through the differentiated ectoplasm of the anterior region have been seen.

The feeding habits of *Trichonympha* are similar to those described above for *Leidyopsis*. A change in the shape of the body occurs but is not so striking as in *Leidyopsis*. The posterior portion only partakes in this change, often losing its elongate rounded form and becoming wider, giving to the flagellate a bell-shape with the lower margin considerably swollen. The long flagella on the posterior portion of the differentiated ectoplasm extend backward with their tips interlacing in the ordinary form. When the lower part of the



Figs. 12–14. *Trichonympha* sp., drawn from living specimens, showing various stages in the ingesting of food bodies. Note short clear pseudopods in figure 12 and the long one in figure 13. × 200.

bell becomes wider they do not form such a close covering of the posterior portion and food particles may be more easily swept into contact with the hungry ectoplasm of that region. The same result is obtained when the posterior portion becomes drawn out beyond the tips of the flagella. It seems probable that this latter is the usual form that the flagellate assumes when actively feeding. In many individuals the posterior portion of the body containing food bodies occupies about one-third to one-fourth the entire length of the body (figs. 12, 13). Individuals may show these proportions reversed. One flagellate was measured which was 560μ in length and only 140μ from the anterior tip to the posterior margin of the nucleus. The remaining portion was literally filled with food particles.

The flagella do not seem to take any part in the capture of food bodies, though it may be that particles becoming entangled in the flagella are more easily captured by the ectoplasm. When contact has been made the same procedure follows as in *Leidyopsis*, the body often becoming distorted in its attempts to take in disproportionately large pieces (fig. 14).

The relatively large amount of food material used by these flagellates may be directly proportioned to their activities, since these exceed, in energy expended in meeting the impact of their environment, the activity of any other forms known to the writer. More remarkable is the method of feeding, since they are flagellates which combine the highest degree of ectoplasmic development to be found in the Protozoa, covering with the cilia-like flagella from one-third to two-thirds or more of the body surface, with the thin mobile ectoplasm characteristic of the Rhizopoda over the remaining portion of the body. The degree of development of the anterior part of the body is undoubtedly the result of environmental conditions (Kofoid, 1923) and, in the absence of a cytostome anteriorly, the amoeba-like ingesting of food at the posterior end seems also to be the result of the same factors.

SUMMARY

Trichonymphid flagellates parasitic in the digestive tract of wood-eating termites do not feed by means of their abundant anterior flagella, nor by means of an anterior gullet as proposed by Kent (1884), nor at any point on the flagellated area as suggested by Porter (1897), but by means of pseudopodia formed on the posterior region of the body. These pseudopodia are peculiary adhesive, clinging to the particle of wood which they touch, and slowly creeping out over these particles until they completely engulf them. Particles double the diameter of the body may be engulfed.

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Transmitted May 1, 1923.

LITERATURE CITED

Kent, S.
1884. Notes on the infusorial parasites of the Tasmanian white ants. Papers and Proc. Roy. Soc. Tasmania, **1884**, 270–273.

Kofoid, C. A.
1923. Parasitism and evolution. Address at National Academy of Sciences, April 23, 1923. MS.

Kofoid, C. A., and Swezy, O.
1919*a*. Studies on the parasites of the termites. III. On *Trichonympha campanula* sp. nov. Univ. Calif. Publ. Zool., **20**, 41–98, pls. 5–12, 4 figs. in text.

1919*b*. Studies on the parasites of the termites. IV. On *Leidyopsis sphaerica* gen. nov., sp. nov. *Ibid.*, **20**, 99–116, pls. 13–14, 1 fig. in text.

Leidy, J.
1881. The parasites of the termites. Jour. Acad. Nat. Sci., Philadelphia, (2), **8**, 425–450, pls. 51–52.

Porter, J. F.
1897. *Trichonympha* and other parasites of *Termes flavipes*. Bull. Mus. Comp. Zool., Cambridge, **31**, 48–68, pls. 1–6.

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METHODS OF OBTAINING AMOEBA-FREE RATS FOR EXPERIMENTAL INFECTION WITH INTESTINAL AMOEBAE

BY
JOHN F. KESSEL

EXPERIMENTAL INFECTION OF RATS AND MICE WITH THE COMMON INTESTINAL AMOEBAE OF MAN

BY
JOHN F. KESSEL

Smithsonian Institution
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METHODS OF OBTAINING AMOEBA-FREE RATS FOR EXPERIMENTAL INFECTION WITH INTESTINAL AMOEBAE

BY
JOHN F. KESSEL

CONTENTS

PAGE
A. Routine examination of normal faeces 401
B. Examination of faeces of rodents after administration of a purgative 403
C. Incidence of infection at different ages 407
D. Conclusions 408
E. Literature cited 408

In an investigation carried on recently by the writer an attempt was made, first, to determine the different species of intestinal amoebae found in culture rats and mice, and second, to establish, in culture rats and mice, experimental infections of the common intestinal amoebae of man and of the amoebae common to rats and mice. During these experiments methods for hastening the evacuation of the intestinal Protozoa in the faeces have been developed which it is thought will prove of value to other investigators in similar fields.

The writer is greatly indebted to Professor C. A. Kofoid of the Department of Zoology in the University of California for suggestions and guidance throughout the whole investigation of which this paper records a part.

A. ROUTINE EXAMINATION OF NORMAL FAECES

Examination of human faecal material for the presence of intestinal Protozoa has shown that it is necessary to examine samples taken on several successive days in order to make accurate and complete diagnosis of the organisms present. No experimental work has as yet been done to determine the degree of certainty of the results of such examinations of human faeces, but statistics show that the

greater the number of samples examined, the greater the percentage of infections found (see Kofoid, 1920). In the Department of Parasitology, California State Board of Health, in Berkeley, six routine examinations of human faeces without finding infection are made before a case is pronounced as probably negative. Dobell (1917 and 1920) has also pointed out the advisability of making six routine examinations before a human case is pronounced uninfected and in 1917 states that "the expectation for the average infected case is that it will be found positive twice in every five examinations."

Routine examination of the normal faeces of rats and mice has shown this method to be less accurate with these animals than is probably the case with man. Two reasons may be given for this fact. First, the number of amoebae found in the rodents is considerably less than is found in man, and, second, the active stages of the intestinal Protozoa found normally in rats and mice inhabit the small intestine and caecum in the main and are seldom found in the large intestine, while in man, amoebic infections, including their cysts, are common in the colon.

Brug (1919) kept records of routine examinations for amoebae in the normal faeces of six rats and concluded that this method is not thoroughly reliable. His results are included in table 1 for the sake of comparison with similar conclusions which were drawn early in the investigation by the present writer.

It will be seen from the accompanying table that only 16.3 per cent of all the examinations made gave positive results, though all the animals examined were known to be infected with amoebae. Brug (1919) states that "the cysts may be found for several days in succession, then for several days, weeks or months in succession nothing may be found." Similar results, but not for intervals so long as months, were obtained in the present investigation. Further, the number of cysts, when present, may vary greatly. At times only one cyst may be found on the entire cover glass preparation, while at other times as many as three or four cysts may be found with the 4 mm. objective in one field of the microscope. As a rule, cysts only were found in the faeces, though on three occasions motile amoebae were present. These facts point to the conclusion that there is no regular periodicity in the discharge of intestinal amoebae in these rodents.

It is evident from these data that the small number of examinations sufficing for the detection of intestinal Protozoa in man would

be inadequate in making a diagnosis for rats and mice. It is further seen that the number of examinations necessary to determine the exact infections present, or to make it relatively certain that an animal is free from a given infection, would extend over an almost prohibitive period of time in experimental work. It was therefore decided to evacuate the amoebae in the faeces through administration of a purgative.

TABLE 1

Results of Routine Examination of Normal Rodent Faeces for Intestinal Amoebae

Rats examined by Brug	Time in months	Number of examinations	Times positive	Times negative	%
Rat No. 1	5	105	8	97	7.6
Rat No. 2	5	88	13	75	14.7
Rat No. 3	3	91	8	83	8.8
Rat No. 4	2	37	7	30	19.0
Rat No. 5	2	26	1	25	3.8
Rat No. 6	2	28	2	26	7.1
Totals		375	39	336	10.6
Rats and mice examined by Kessel					
Rat No. 1	3	22	9	13	40.9
Rat No. 2	2	10	1	9	10.0
Rat No. 3	3	22	10	12	45.5
Rat No. 4	2	28	2	26	7.1
Rat No. 5	2	28	9	19	32.1
Mouse No. 1	2	15	6	9	40.0
Mouse No. 2	2	14	6	8	43.0
Mouse No. 3	2	14	4	10	28.6
Totals		153	47	106	30.6
Grand totals		528	86	442	16.3

B. EXAMINATION OF FAECES OF RODENTS AFTER ADMINISTRATION OF A PURGATIVE

Castor oil mixed with food was first tried as a purgative. The faeces were evacuated successfully, but the oil droplets in the smear were confusing because of their resemblance, in optical properties, to amoebic cysts, especially when seen through a low power mieroscope. So commercial epsom salt was used instead. At first this was mixed with the drinking water and given to the rats in their water bottles. As the animals refrained from drinking the mixture, except when especially thirsty, the salt was mixed with food.

TABLE 2

RESULTS OF DOSING RATS WITH MAGNESIUM SULPHATE PRIOR TO EXAMINATION FOR INTESTINAL PROTOZOA

Infections by	Faecal Examinations Days		Examinations at Autopsy						Remarks
			Large Intestine	Caecum	Small Intestine		Total Positive	Total Negative	
	1st	2d			Ileum	Duodenum			
Amoeba	10	....	10	13	0	0	13	53	Examined one time only. Did not note flagellates
Amoeba	9	8	17	18	2	0	18	44	
Total amoebae	19	8	27	31	2	0	31	97	Infections in caecum only were very light
Trichomonas	40	13	53	57	3	0	57	5	
Hexamitus	4	7	11	12	?	19	19	43	
Giardia	2	3	5	6	11	15	15	47	
Chilomastix	14	2	16	17	?	?	17	45	

Stale bread was soaked in a solution of water saturated with the magnesium sulphate. Hungry rats usually ate this mixture readily, though the mice often refused to eat it until they had been offered nothing else for several days. If the rats ate readily of the bread and salts at 6 P.M., a sufficient quantity of semi-fluid material was assured for examination during the next day. Rats which gave no reaction from the salt the morning after being fed the prepared bread were placed together in a separate cage and redosed, and were regarded as unexamined rats. Rats which gave a positive reaction to the purgative were given a lighter dose of salt the second night. A second examination of faecal material was made the following day, after which the animals were autopsied and examination was made of the intestinal contents at different levels of the digestive tract.

In making the smears a drop of normal salt solution was placed near one end of the slide and about half an inch toward the middle of the slide was placed a drop of Donaldson's iodine-eosin stain. The faecal material was then smeared, first in the saline portion and then in the iodine-eosin portion. In placing the cover glass on the smear, contact was made first with the saline half of the smear. The motile forms are easily detected in the normal saline solution and the cysts more easily seen in the iodine-eosin. Cover glasses 12 mm. square were used and two smears were made on the same slide. A 5× ocular was used first with a 16 mm. objective for determining the presence of the organisms. Then the 4 mm. objective was used for more detailed study and for determining the species of organism present.

As the writer's investigation concerned amoebae only, no account was taken of other Protozoa in the first series of animals examined. In the second series examined other Protozoa were noted when present. Results from the examination of 128 rats are shown in table 2.

From the above table it will be noted that in one hundred and twenty-eight rats examined, amoebae were found in the caecum in only four rats where they had not been detected previously in the faeces. Three of these cases had been examined on one day only and the amoebae probably would have been detected had a second examination been made. The fourth infection in the caecum only was a very light infection. The four cases of *Trichomonas* found in the caecum only were also exceptionally light infections. Amoebae were found twice in the ileum while *Trichomonas* was found four

times. The numbers found were small and it is probable that these may have been forced from the caecum by the peristalsis induced by the purge.

It should be noted here that during this investigation rats which were known to harbor infections of amoebae and which showed no amoebae in faeces collected without the administration of a purge, invariably showed amoebae in the caecum at autopsy, which followed immediately after faecal examination, though amoebae were not found in the colon at autopsy.

The general conclusions to be drawn from the above facts are as follows:

1. Faecal examination of rats on two consecutive days after they have been purged with epsom salt is a comparatively safe method for detecting the presence of amoebae and *Trichomonas muris* in these animals.

2. Amoebae common to rats and mice and *Trichomonas muris* commonly inhabit the caecum of these animals.

3. *Giardia muris*, *Hexamitus muris*, and probably *Chilomastix* inhabit the small intestine normally, and their presence in rodents cannot always be detected upon examination of the faeces after administration of epsom salt.

Hegner (1923) has found a much higher percentage of rats infected with *Giardia muris* and *Hexamitus muris* than has been found in this laboratory. It thus seems that the incidence of infection for different Protozoa may vary greatly in different rodent colonies.

During the present investigation it has been found that it is much more difficult to work with mice than with rats. In the first place, mice will not eat the mixture of bread and salts so readily as the rats will, and in the second place, it is more difficult to collect semi-liquid faeces from mice than from rats. No table to determine the accuracy of the epsom salt method for examining mice for Protozoa has been compiled, but from a few examinations made it is concluded that on account of the difficulty encountered in working with mice the method cannot be relied upon as it can in the case of rats.

C. INCIDENCE OF INFECTION AT DIFFERENT AGES

In choosing animals for experimental infections it is advisable to select those most susceptible to infection. The present investigation has shown that the incidence of amoebic infection among culture rats in this laboratory is 34 per cent. The rate of infection is much higher in middle-aged animals than it is in very young animals or in very old animals as is shown in table 3.

TABLE 3

INCIDENCE OF INFECTION OF AMOEBAE IN CULTURE RATS AND MICE AT DIFFERENT AGES

Age	Mice			
	Positive	Negative	Total	% Positive
Under two months	2	7	9	22 0
Two to four months	16	15	31	51 6
About ten months	12	28	40	30 0
Age	**Rats**			
	Positive	Negative	Total	% Positive
Under two months	9	44	53	17 0
Two to ten months	54	42	96	56 3
Over ten months	3	40	43	7 0

From this table it is seen that animals between the ages of two and ten months show by far the highest rate of infection. The inferences are that young animals, before weaning, either because of their milk diet (Kessel, 1923*b*) or because of the minimum degree of exposure to infection, have not established an infection; that the older animals have, in some way and to some extent, cleared themselves of infection; that the animals midway between these two ages not living on a diet of milk exclusively, are subject to constant exposure, and, since they have not had time to establish an immunity, show the highest degree of infection.

In this investigation, in most cases, old amoeba-free animals failed to become infected when fed cysts by mouth, while young amoeba-free animals fed on the same material established an infection (see Kessel, 1923*a* and *b*).

It may therefore be concluded that the most advantageous time to choose rats for feeding experiments is shortly after weaning or at about the age of two months. Among rats of this age will be found the greatest number free from amoebic infection and also those rats with the greatest susceptibility to infection.

D. CONCLUSIONS

1. Routine examination of normal rodent faeces for discovering intestinal Protozoa is a tedious and unreliable method.

2. Stale bread soaked in a saturated solution of magnesium sulphate in water is a convenient purgative to administer to rats.

3. Examination on two consecutive days of the faeces from 128 purged rats, procured by this method, after which the rats were autopsied and the intestinal contents examined, showed that this is a relatively safe method for the detection of amoebic and trichomonad infections in rats.

4. Young rats show the greatest susceptibility to infection and are the most satisfactory for infection experiments.

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E. LITERATURE CITED

Brug, S. L.
1919. De entamoeben van de rat. Geneesk. Tijd. Nederl. Indië, **59**, 1–15, pls. 1–2.

Dobell, C.
1917. Reports upon investigation in the United Kingdom of dysentery cases received from the Eastern Mediterranean. I. Amoebic dysentery and the protozoological investigation of cases and carriers. [British] Medical Research Committee, Special Report Series, No. 4 (London, Joseph Causton and Sons, Ltd.), pp. 1–85.

Dobell, C., and O'Connor, F. W.
1921. The intestinal Protozoa of man (London, John Bale, Sons and Danielsson, Ltd.), v + 211 pp., pls. 1–8, 2 figs. in text.

Hegner, R. W.
1923. The effects of changes in diet on the incidence, distribution and numbers of certain intestinal Protozoa of rats. Am. Jour. Hyg., **3**, 180–200, pl. 4.

Kessel, J. F.
1923*a*. Experimental infection of rats and mice with the common intestinal amoebae of man. Univ. Calif. Publ. Zool., **20**, 409–430, pls. 38–39.
1923*b*. The distinguishing characteristics of the parasitic amoebae of culture rats and mice. *Ibid.* (*In press.*)

Kofoid, C. A., and Swezy, O.
1920. On the prevalence of carriers of *Endamoeba dysenteriae* among soldiers returning from overseas service. New Orleans Med. and Surg. Jour., **73**, 4–11.

Kofoid, C. A., Kornhauser, S. I., and Swezy, O.
1919. Intestinal parasites in overseas and home service troops of the U. S. Army. Jour. Am. Med. Assoc., **72**, 1721–1724.

Wenyon, C. M.
1907. Observations on the Protozoa in the intestine of mice. Arch. f. Prot., Suppl. **1**, 169–201, pls. 10–12.

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EXPERIMENTAL INFECTION OF RATS AND MICE WITH THE COMMON INTESTINAL AMOEBAE OF MAN

BY

JOHN F. KESSEL

CONTENTS

PAGE

A. Introductory and historical . . . 410
B. Feeding experiments . . . 411
I. Materials and methods . . . 411
II. Tables and conclusions . . . 412
Table 1. Rats fed with *E. dysenteriae* . . . 413
Table 2. Mice fed with *E. dysenteriae* and *C. lafleuri* 414
Table 3. Rats fed with *E. coli* . . . 415
Table 4. Rats fed with *C. lafleuri*, *E. nana*, and *I. bütschlii* 416
Table 5. Rats fed with *E. coli*, and *C. lafleuri* . . . 417
Table 6. Rats fed with *E. dysenteriae*, *E. coli*, and *C. lafleuri* obtained from other rats . . . 419
III. Effect on cysts of passage through digestive tract . . . 419
Table 7. Results from autopsying rats and mice fifteen hours after feeding cysts of *E. dysenteriae* and *C. lafleuri* . . . 420
C. Discussion . . . 421
I. Specificity of parasites to a given host . . . 421
II. Factors involved in establishment of a parasitic infection by amoebae 422
III. Application of cross-infection experiments from the point of view of medical zoology . . . 423
IV. Morphological constancy of parasites in cross-infection experiments . . . 424
D. Summary . . . 424
E. Literature cited . . . 426
F. Plates and explanations . . . 428

A. INTRODUCTORY AND HISTORICAL

The present investigation was first attempted to determine whether or not rats and mice could be experimentally infected with *Endamoeba dysenteriae* (Councilman and Lafleur). Most of the attempts to infect rodents by feeding them with cysts of *E. dysenteriae* recorded in literature prior to this time have been unsuccessful (see Kruse and Pasquale, 1894; Werner, 1908; Dale and Dobell, 1917; Wenyon and O'Connor, 1917; and Chatton, 1917, 1918).

Lynch (1915), however, has recorded spontaneous and experimental infection of rats in Charleston, South Carolina. He made his diagnosis of amoebae entirely from the motile forms and took no note of the fact that there are intestinal amoebae normal to rats and mice, the motile forms of which might easily be confused with *E. dysenteriae*, as both have hyaline pseudopodia. For an account of the amoebae found in rats and mice see Grassi (1881), Wenyon (1907), Brug (1919), Rudovsky (1921), and Kessel (1923*b*). See Wenyon and O'Connor (1917) and Dobell (1919) for an account of the pseudopodia of *E. dysenteriae*. Lynch published no figures and while the clinical symptoms he describes indicate the presence of acute infections of *E. dysenteriae*, it seemed possible that his rats might have died of some other infection common to rats, as implied in the criticisms of Chatton (1917) and of Dobell (1919).

In 1919, Brug reported what he thought was an infection of *E. dysenteriae* in two wild rats in Java and further concluded that he had been successful in establishing an experimental infection of *E. dysenteriae* from man in a rat of the species *Mus rattus*. His work seems to be conclusive, yet he entertains some doubt as to whether the species he found in the rats and *E. dysenteriae* of man are identical.

Baetjer and Sellards (1914) reported successful inoculation of guinea-pigs with *E. dysenteriae*, lesions of the bowel being found at autopsy. Chatton (1918) concluded that guinea-pigs may be infected with *E. dysenteriae* either by feeding cysts or by anal injections and that the infections, once established, are restricted to the caecum.

Huber (1909) succeeded in infecting rabbits by feeding them cysts of *E. dysenteriae* and the examination of the rabbits at autopsy showed a localization of amoebic ulcers in the caecum.

The species of intestinal amoebae common to man, other than *E. dysenteriae*, have never been experimentally established in rats and mice. The present investigation also included an attempt to infect these rodents with *Councilmania lafleuri* Kofoid and Swezy and to infect rats with *E. coli* (Lösch), *Endolimax nana* (Wenyon and O'Connor), and *Iodamoeba bütschlii* (Prowazek).

It was apparent from the first that a detailed study of the amoebae normal to the rat and mouse was essential before critical experimental infections could be carried out satisfactorily. Accordingly, this work was undertaken first, and to date three species of intestinal amoebae, *Councilmania muris* (Grassi) Kessel, *Councilmania decumani* (Rudovsky) Kessel, and *Endamoeba ratti* sp. nov., have been found in rats and mice and conclusions relating to their distinguishing characteristics have been recorded in a separate paper (Kessel, 1923*b*).

For assistance and guidance in the present work the writer is glad to make acknowledgment to Professor C. A. Kofoid, under whose supervision the investigation has been carried on, to Professor E. L. Walker, who has been kind in giving counsel and advice, and to Dr. Olive Swezy, whose suggestions have been of great value. Miss Inez Smith, technician of the Division of Parasitology, California State Board of Health, has generously coöperated in supplying material from that laboratory for use in the feeding experiments.

B. FEEDING EXPERIMENTS

I. Materials and Methods

The rats used for the feeding experiments have been obtained from the rat colony of the Department of Zoology, University of California, and belong to the species *Rattus norvegicus* (Erxleben). For a history of the colony, see Kessel, 1923*b*.

The rats chosen for experimental infection were young rats, about two months of age. It was found during the experiment that male rats are more suited to this type of work since it is easier to collect faeces from them than from the females.

Rats known to be free from amoebic infection of any type, and other rats known to harbor an infection of *Councilmania* common to the rat, were used. Amoeba-free rats were isolated by the epsom salt method described by Kessel (1923*a*). These rats were kept separate from those known to be infected with amoebae.

The material used for feeding was obtained from the laboratory of the Division of Parasitology of the California State Board of Health, which is under the direction of Professor C. A. Kofoid. The diagnoses were made first by that laboratory and later authenticated by the writer before feeding the rats. The morphological characteristics of the amoeba common to man were compared with the descriptions and figures of Kofoid, Kornhauser, and Swezy (1919), Dobell (1919), Kofoid and Swezy (1919), and Kofoid (1923). As it was not always possible to obtain a sufficient quantity of faecal material to feed a large number of rats, the interval between feeding in some instances is considerable. In feeding, bread was soaked in water until it became quite soft. The water was then squeezed out and a small handful of bread was placed in each feeding dish. The faecal material was well mixed with water in another dish until it was very liquid in consistency. A portion of this was then poured over the bread in each dish and the contents well stirred. There was no constant proportion of faecal material to bread, for the amount of faecal material obtainable varied. The rats ate well of all mixtures fed even though the bread was always colored by the faeces.

After the feeding experiments were completed and an interval of two weeks or longer had passed, the rats were examined, always after being given a dose of epsom salt. Fresh smears were examined in iodine-eosin and permanent smears were stained with iron haematoxylin.

II. Tables and Conclusions

The results of the feeding experiments are displayed in tables, and a short discussion follows of the conclusions drawn from each table.

Discussion and conclusions from table 1

1. Eight out of twenty-nine, or 27.6 per cent of *all* the rats fed, became infected with *E. dysenteriae*.

2. Eight out of ten, or 80 per cent of all *amoeba-free* rats fed, became infected with *E. dysenteriae*. This may have been due in part to their being non-infected rats, and in part to their having reeeived an extra feeding of *E. dysenteriae* cysts on January 5 which the other rats did not receive. It will be noticed in tables 4 and 5 that a greater percentage of amoeba-free rats became infected than was the case with rats having a previous amoebic infection with rat amoebae.

3. Of the eleven rats that became infected with *E. dysenteriae* (see also table 4), four died between the time of the last feeding, January 17, and June 12.

TABLE 1

RESULTS OBTAINED FROM FEEDING RATS WITH CYSTS OF ENDAMOEBA DYSENTERIAE

Rat Infection not known	Feedings with cysts of *E. dysenteriae* from man	Examinations May 16, 18, 21, 22, 23, 25, 26 and June 3				Autopsy June 4	Remarks
No. 1	April 16, 18, and 21, 1921	0				0	
2		0				0	
3		0				0	
4		0				0	Motile *Trichomonas*, smaller than *Tritrichomonas* of rat, apparently *Trichomonas hominis*.
Amoeba-free		**Jan. 31**	**Feb. 28**	**April 1**	**June 8**		
No. 5		0	0	0	0		
6		+	+	+	+		
7		+	+	+	+		
8	Oct. 20, 1922 Nov. 1, 1922	+	+	−	−	+	Bloody mucus in stool Jan. 31. Died Mar. 2 of lung infection. Had large ulcer of colon.
9	Jan. 2, 1923	+	+	+	+		
10	Jan. 5, 1923 Jan. 17, 1923	+	+	+	−	+	Bloody mucus in stool Jan. 31.
11		+	+	+	−	+	Died May 20 of lung infection.
12		0	0	0	0		
13		+	+	+	+		
14		+	+	+	+		
Infected with amoebae of rat							
Nos. 15 to 29	Oct. 20, 1922 Nov. 1, 1922 Jan. 2, 1923 Jan. 17, 1923	0	0	−	−	0	None of the rats previously infected with amoebae common to the rat became infected with *E. dysenteriae*. The infection of rat amoebae remained positive in ten of the fifteen cases.

4. For the most part no tendency to diarrhoea was noticed among the rats; on the contrary the faeces were harder than normal, thus showing a condition of constipation.

5. The four rats that died gradually became thin and the hair was ruffled several days before death. An intestinal ulcer was found in the colon of No. 8 while in Nos. 10, 11, and 62 no lesions were found, though a mucoid condition was noticeable. In each case there

was a severe lung infection which was the apparent cause of the death of the rats. Smears and sections made from the infected parts of the lungs revealed no amoebae.

6. The type of lung infection found in these rats is the most common cause of death among rats in the rat colony in the Department of Zoology. During the period in which the four of the eleven rats infected with *E. dysenteriae* died, one rat infected with *E. coli* and three from the colony of one hundred and fifty uninfected rats died of the same lung disease. Thus the mortality among the eleven rats infected with *E. dysenteriae* was four and the mortality among all the other experimental and uninfected rats of the rat colony, or a total of two hundred and twenty-five rats, was also four. It seems probable that the resistance of the rats infected with *E. dysenteriae* was so lowered that they were more susceptible to the lung infection. It is significant in this connection to note that a rabbit fed cysts of *E. dysenteriae* by Chatton (1918) died of a lung infection.

7. The course of the infection of *E. dysenteriae* among the rats in this experiment was the chronic rather than the acute type which seems to be the common type of amoebic infection found in kittens. Seven of the rats infected with *E. dysenteriae* were in apparent health at the time the last examination was made, though they all showed the presence of cysts.

8. Motile amoebae, mononucleate cysts, binucleate cysts, and four-nucleate cysts of *E. dysenteriae* were recovered from the rats, and these were without any apparent morphological or racial change from their previous status in man (pl. 38, figs. 7–12).

TABLE 2

Results Obtained from Feeding White Mice with Cysts of E. dysenteriae and of Councilmania lafleuri

Mouse Adult, amoeba-free	Feedings	Examinations			Autopsy	Remarks
		Feb. 3	Feb. 9	Feb. 18	Mar. 2	
No. 1	January 11, 13 and 14	0	0	0	0	
2		0	0	0	0	
3		+	+	+	+	Cysts and motile
4		0	0	0	0	*E. dysenteriae.* Mouse normal.
5		0	0	0	0	
6		0	0	0	0	
7		+	+	+	+	Cysts and motile
8		0	0	0	0	*Councilmania lafleuri.* Mouse
9		0	0	0	0	normal.
10		0	0	0	0	

DISCUSSION AND CONCLUSIONS FROM TABLE 2

1. As no young animals were available for this experiment, only adult amoeba-free mice were used.

2. One mouse only became infected with *E. dysenteriae* and only one with *Councilmania lafleuri*.

3. As shown by Kessel (1923*b*) adult animals are more difficult to infect with amoebae than young ones, and it is probable that the low percentage of infection in this series of amoeba-free mice is due to the advanced age of the animals. This indicates that in some cases a resistance or immunity to infection is established in old animals.

4. The cysts recovered were apparently normal and the species had undergone no morphological change though passed through the rodent host (pls. 38–39).

TABLE 3

RESULTS OBTAINED FROM FEEDING RATS WITH CYSTS OF ENDAMOEBA COLI

Rat	Feedings of cysts of *E. coli* from man	Examinations		Autopsy	Remarks
Infection unknown		Jan. 18	Jan. 25	Jan. 29	
No. 30		+	+	+	*E. coli*
31		+	+	+	*Councilmania sp.* of rat
32		+	+	+	Mixed
33		+	+	+	*E. coli*
34		+	+	+	*Councilmania sp.* of rat.
35	Dec. 19	+	+	+	*E. coli*
36	Jan. 8	0	+	+	*Councilmania sp.* of rat
37		0	0	0	
38		0	0	0	
39		+	+	+	*E. coli*

DISCUSSION AND CONCLUSIONS FROM TABLE 3

1. No amoeba-free rats were on hand when this feeding experiment was begun, so young rats were chosen, the infection of which was unknown. As they had only recently been weaned it is probable that the percentage of infection was low.

2. The *Councilmania* found in the final examinations was in each case one of the species, at that time undetermined, common to the rat, and it is concluded the rats had this infection when fed with *E. coli*.

3. An infection of *E. coli* only was recovered from four of the ten rats, while a mixed infection of *E. coli* and *Councilmania* common to the rat was found in one rat. Thus five out of ten or 50 per cent of the rats fed with *E. coli* established an infection.

4. The motile forms showed characteristic granular pseudopodia and the cysts were identical in size and morphological characteristics with the cysts fed to the animals (pl. 38, fig. 3).

TABLE 4

Results Obtained from Feeding Rats with Cysts of Councilmania lafleuri, Endolimax nana, Iodamoeba bütschlii, and Endamoeba dysenteriae

Rats Amoeba-free	Feedings	Examinations Jan. 23	Feb. 24	June 8	Autopsy	Remarks
No. 40		+	+	+	+	*Councilmania lafleuri*, *Endolimax nana*, *Iodamoeba bütschlii*
41		0	0	0	0	
42		0	0	0	0	
43		+	+	+		*C. lafleuri*
44		+	+	+	+	*C. lafleuri*
45		+	+	+	+	*C. lafleuri*
46		+	+	+		*C. lafleuri*
47		+	+	+		*C. lafleuri*
48		0	0	0	0	
49		+	+	+		*E. dysenteriae*, *Endolimax nana*, *Iodamoeba bütschlii*
50		+	+	+	+	*C. lafleuri*
51		+	+	+		*C. lafleuri*
52	November 14	+	+	+		*C. lafleuri* and *Giardia*
53	December 2	+	+	+		*C. lafleuri*
54	January 5	+	+	+		*E. dysenteriae*
Infected with amoeba of rat						
No. 55		0	0	0		
56		0	0	0		
57		0	0	0		
58		0	0	0		
59		0	0	0		
60		+	+	−		*C. lafleuri*
61		0	0	0		
62		+	+	+	+	*E. dysenteriae*
63		0	0	0		Died of lung infection, June 12
64		0	0	0		

DISCUSSION AND CONCLUSIONS FROM TABLE 4

1. Eleven of the twenty-five rats, or 44 per cent, fed with cysts of *Councilmania lafleuri* became infected.

2. Ten of the fifteen amoeba-free rats, or 66 per cent, fed with cysts of *Councilmania lafleuri* became infected.

3. Two of the twenty-five rats, or 8 per cent, fed with cysts of *Endolimax nana* and *Iodamoeba bütschlii* became infected.

4. Cysts of *Edamoeba dysenteriae* were recovered from three rats of this series, although the presence of such cysts had been overlooked, but later found, in the stained slides of the human faecal material fed to these animals. A light infection in man was thus detected by the feeding experiment.

TABLE 5

RESULTS OBTAINED FROM FEEDING RATS WITH CYSTS FROM A MIXED INFECTION OF ENDAMOEBA COLI AND COUNCILMANIA LAFLEURI

Rats	Feedings	Examinations			Autopsy	Remarks
Amoeba-free		Jan. 23	Jan. 24	Feb. 7		
No. 65	December 12 and 14	+	+	+	+	*C. lafleuri*
66		+	+	+	+	Mixed *E. Coli* and *C. lafleuri*
67		+	+	+	+	Mixed *E. coli* and *C. lafleuri*
68		0	0	0	0	
69		0	0	0	0	
70		+	+	+	+	*C. lafleuri*
71		+	+	+	+	*C. lafleuri*
72		+	+	+	+	*C. lafleuri*
73		+	+	+	+	Mixed *E. coli* and *C. lafleuri*
74		+	+	+	+	*C. lafleuri*
Infection unknown						
No. 75		0	0	0	0	
76		+	+	+	+	*C. lafleuri*
77		+	+	+	+	*E. coli*
Nos. 78 to 85		0	0	0	0	
No. 86		+	+	+	+	*E. coli*
87		+	+	+	+	*E. coli*
88		0	0	0	0	
89		0	0	0	0	

5. Of the three rats infected with *E. dysenteriae*, one died of a lung infection on June 12. The other two on that date showed cysts in the faeces but were in apparent normal health.

6. The faeces containing the cysts of *E. dysenteriae* were fed to the rats January 5. As the rat faeces contained cysts of *E. dysenteriae* in fair numbers on January 23, or eighteen days later, it may be concluded that an infection of *E. dysenteriae* may be established in rats within eighteen days after feeding them with the amoeba cysts. It is highly probable that the infection was well established several days before this time though an examination was not made.

7. A *Giardia*, resembling *Giardia enterica*, was recovered from one of the rats in this series. As no cysts of this flagellate had been detected in the feeding material, the infection in the stools used must have been very light.

8. The cysts of *Councilmania lafleuri, Endolimax nana, Iodamoeba bütschlii*, and *Endamoeba dysenteriae* recovered from this series of rats were without any apparent racial or morphological change.

9. The rats which became infected with *C. lafleuri, Endolimax nana*, and *Iodamoeba bütschlii* remained in apparent normal health.

DISCUSSION AND CONCLUSIONS FROM TABLE 5

1. Twelve out of twenty-five, or 48 per cent of the rats fed a mixed infection of *E. coli* and *Councilmania lafleuri*, became infected with both or one of these amoebae.

2. Eighty per cent of the amoeba-free rats became infected while only 27 per cent of the rats of unknown previous infection acquired the amoebae. It is probable that the ones that became infected were amoeba-free at the time of feeding.

3. The motile amoebae of *C. lafleuri* were easily distinguishable by their hyaline pseudopodia while the motile amoebae of *E. coli* showed the characteristic granular pseudopodia. The cysts of both species were also morphologically characteristic after being passed through the rat, and presented no apparent structural change.

DISCUSSION AND CONCLUSIONS FROM TABLE 6

1. *E. dysenteriae, E. coli*, and *C. lafleuri*, respectively, may be transferred from rats experimentally infected with each of these amoebae to uninfected rats.

2. The cysts and motile forms of the amoebae may be recovered without any apparent morphological change after transfer.

3. None of the rats infected in this experiment suffered any apparent pathogenic consequences during the time of the experiment. This indicates that amoebae that have passed through a second series of rats are no more virulent than those passed directly from man to rats.

III. Effect on Cysts of Passage Through Digestive Tract

During this investigation the faecal material of the rats and mice has been examined on a number of occasions twenty-four and forty-eight hours after feeding the animals with human faecal material. There is no constancy as to the appearance of normal or disintegrated

TABLE 6

Results Obtained from Feeding Cysts of E. dysenteriae, E. coli, and Councilmania lafleuri, Respectively, Obtained from Infected Rats to Other Amoeba-Free Rats

Rats Amoeba-free	Material	Feedings	Examinations April 21	Examinations May 3	Examinations May 4	Remarks
No. 90	*E. dysenteriae* from Rat No. 10	Faecal material fed daily from Feb. 19 to April 15	0	0	0	
91			0	0	0	
92			+	+	+	Cysts only
93			+	+	+	Motile forms and cysts
94			0	0	0	
95	*E. coli* from Rat No. 86		+	+	+	Motile amoebae
96			+	+	+	Motile amoebae and cysts
97			+	+	+	Motile amoebae and cysts
98			0	0	0	
99			+	+	+	Motile amoebae
100	*C. lafleuri* from Rat No. 40		+	+	+	Motile amoebae and cysts
101			0	0	0	
102			+	+	+	Motile amoebae and cysts
103			+	+	+	Motile amoebae and cysts
104			+	+	+	Motile amoebae and cysts

human amoebic cysts in the rat faeces. Cysts which are apparently unmolested by the passage through the rodent have been recovered while other cysts in different stages of disintegration have been detected. In the examinations made 44 per cent contained no cysts of any type, 40 per cent contained dead or degenerate cysts, and 16 per cent contained live cysts. This indicates a difference in viability of the cysts.

Two young amoeba-free rats and three young amoeba-free mice were fed a mixture of barium sulphate, milk, and human faecal

TABLE 7

RESULTS FROM AUTOPSYING RATS AND MICE FIFTEEN HOURS AFTER FEEDING THEM WITH CYSTS OF E. DYSENTERIAE AND C. LAFLEURI

Animal	Faeces		Colon		Caecum		S. Intestine	
	E. dysenteriae	*C. lafleuri*	*E. dysenteriae*	*C. lafleuri*	*E. dysenteriae*	*C. lafleuri*	*E. dysenteriae*	*C. lafleuri*
Rat 105	degenerate and normal cysts	0	degenerate and normal cysts	rounded, motile amoebae	degenerate and normal cysts	rounded, motile amoebae	degenerate and normal cysts	0
Rat 106	degenerate and normal cysts	0	degenerate and normal cysts	0 motile amoebae	motile amoebae apparently of both species	motile amoebae apparently of both species	degenerate and normal cysts	normal cysts
Mouse 11	degenerate and normal cysts	degenerate and normal cysts	degenerate and normal cysts	degenerate and normal cysts	normal cysts	normal cysts, one with bud, only one nucleus left in cyst	normal cysts	0
Mouse 12	degenerate and normal cysts	dead cysts	degenerate and normal cysts	dead cysts	rounded, motile amoebae	rounded, motile amoebae	normal cysts	normal cysts
Mouse 13	degenerate and normal cysts	0	0	0	0	0	0	0

material containing cysts of *E. dysenteriae* and *C. lafleuri.* It was possible, on account of the presence of the barium sulphate, to determine that the substance fed passed to all parts of the digestive tract. The animals were autopsied fifteen hours after feeding and examinations were made of the intestinal contents at different levels. The table on page 420 records the results.

DISCUSSION AND CONCLUSIONS FROM TABLE 7

1. Both dead and normal cysts of human intestinal amoebae may be found shortly after feeding in any part of the intestinal tract of the experimental rats and mice.

2. Motile amoebae were found most commonly in the caecum, none being found in the small intestine. Motile forms were found only once in the colon. This indicates that the caecum is the region where the amoebae escape from the cyst, especially as a budding cyst of *Councilmania lafleuri* was found here, there being but one nucleus left in the cyst.

3. The motile amoebae encountered varied greatly in size and were rounded up in most instances. They often presented an uneven margin and abnormal vacuolation, indicating that disintegration was in progress. It is probable that this apparent excessive rate of disintegration of motile amoebae is the result of the process of adjustment to the environment of the new host. Some normal amoebae were found.

C. DISCUSSION

I. Specificity of Parasites to a Given Host

In the history of parasitology two opposing opinions have been evident; one, to regard parasites as being restricted to specific hosts, and the other, to regard as possible in nature the transfer of a parasite from one species of host to another. The first tendency has led to the formation of a great many new species on the basis of their habitat and has minimized, in the minds of the scientific world, the danger of transference of parasite from host to host. Until recently, the second tendency has been greatly underestimated, much of the experimental work recorded indicating that the transference of a given parasite from one species of host to another is often difficult to accomplish.

Certain experimental studies cited in this paper, together with the results of this investigation, lead to the conclusion that transfer is possible at least in certain instances or *under given conditions.* With reference to *E. dysenteriae*, all attempts prior to 1915 to infect rats with this species of amoeba failed. In 1915 Lynch appears to have succeeded in infecting rats with *E. dysenteriae.* Since 1915 four other attempts have been made to infect rats and mice with this same species of amoeba, but, prior to the present investigation, only one has been successful, and that with one rat only. It seems likely that the failures have been due to using an insufficient number of animals, especially of young, amoebae-free individuals.

In the present investigation successful infection of rodents with *E. dysenteriae* has been accomplished in thirteen rats and one mouse, with *E. coli* in seventeen rats, with *Councilmania lafleuri* in twenty-three rats and one mouse, with *Endolimax nana* in two rats, and with *Iodamoeba bütschlii* in two rats (pls. 38–39).

This successful transference to rodent hosts of the amoebae commonly parasitic in the digestive tract of man is evidence in favor of a certain degree of promiscuity of infection of a given species of parasite in several species of host, provided the conditions for successful infection are met.

II. Factors Involved in Establishment of Parasitic Infection by Amoebae

The varying factors in establishing an infection of amoebiasis in the rodent host appear to be (1) the viability of the cysts used in feeding, and (2) the age of the rodent host, together with (3) its active resistance to an amoebic infection.

In some cases large numbers of living amoebic cysts were fed to young rats without successful infection, e.g., Rats 1, 2, 3, and 4 in table 1, while in other cases small numbers of amoebic cysts were fed to a series of rats one time only and successful infection resulted, e.g., Rats 49, 54, and 62. It seems probable that the cysts in the second case were more viable than those in the first case.

Young amoeba-free rodents have acquired an amoebic infection in 80 per cent of the cases fed, while young rodents known to harbor an amoebic infection of amoebae normal to rats and mice have acquired a further amoebic infection in only 15 per cent of the cases. Thus, young amoeba-free rodents are more susceptible to amoebic infection than young rodents with a known amoebic infection.

Young amoeba-free rodents have acquired an infection of amoeba in 80 per cent of the cases fed, while old amoeba-free rodents have acquired an infection in only 20 per cent of the cases. This, together with the facts that 27 per cent of rats of known amoebic infection have later been known to rid themselves of the infection (Kessel, 1923*b*) and that the rate of amoebic infection is much lower in old rodents than in middle-aged rodents (Kessel, 1923*a*), indicates that as the animals progress in age they are able to establish an active resistance to amoebic infection.

III. Application of Cross-Infection Experiments from the Point of View of Medical Zoology

The fact that the amoebae common to the intestinal tract of man have been successfully established in rats and mice is important, first, from the standpoint of preventive medicine. Infection of rats and mice with the amoebae of the human digestive tract has been successful in 55 per cent of all the attempts made, while the cross-infection of these rodents with amoebae common to themselves has been successful in 53 per cent of all the attempts made. In the experimental work of this investigation we thus see that even slightly greater success has attended attempts to infect rats with amoebae common to man than with the amoebae common to rodents (see Kessel, 1923*b*).

The fact of transfer of human infections to rats and mice throws heavy suspicion upon these rodents as facultative carriers of the causative organisms of amoebiasis in man and supports the findings of Lynch (1915) and Brug (1919). Whether the amoebae thus experimentally infected into rodents still retain their pathogenicity for man, it is still impossible to state, but the fact that they may be passed from one rodent to another indicates that the viability of the amoebae is not destroyed by passage through one rodent, and that an infection once established among these rodents may spread to other rodents.

The second important application to experimental medicine of facts determined in this investigation is that the young rats may be used successfully in further investigation relating to pathological conditions resulting from amoebiasis. Experimental infection of kittens with *E. dysenteriae* has resulted in an acute form of amoebiasis (Dobell, 1917) and the amoebae recovered are thought to be abnormal

in appearance. As our rats have carried the infection of *E. dysenteriae* for five months, it seems highly probable that amoebiasis in rats is inclined to become chronic, as it is in man.

IV. Morphological Constancy of Parasites in Cross-Infection Experiments

While many protozoologists hold to the theory that the different species of amoebae common to the digestive tract of man are constant in their morphological characters, respectively, yet there is an idea among certain pathologists that the morphological characteristics of amoebae may alter as the amoebae are transferred from one environment to another.

During this investigation *Endamoeba dysenteriae, Endamoeba coli, Councilmania lafleuri, Endolimax nana,* and *Iodamoeba bütschlii* have been successfully transferred from man to rodents. During the period of infection, which was five months in the case of *E. dysenteriae* and four months in the case of the other species of amoebae, no perceptible morphological change was noted in any of the species used in the experiments. These facts afford valuable evidence in favor of the morphological constancy of given species of amoebae, at least of the failure of immediate change through transfer.

D. SUMMARY

1. Infections of five species of human intestinal amoebae, *Endamoeba dysenteriae, Endamoeba coli, Councilmania lafleuri, Endolimax nana,* and *Iodamoeba bütschlii* have been experimentally transferred to rats by feeding them human faeces containing cysts of these amoebae.

2. Infections of *E. dysenteriae* and *C. lafleuri* have been experimentally transferred to mice by feeding them human faeces containing cysts of these amoebae.

3. The percentage of rodents that have become infected with these five amoebae common to man, is greater among young amoeba-free animals than among old amoeba-free animals, or among young animals known to possess an infection of amoeba common to the rat and mouse.

4. Infections of *E. dysenteriae, E. coli,* and *C. lafleuri* have been experimentally transferred from rats harboring experimental infections of these amoebae to other young amoeba-free rats by feeding them the faeces of the infected rats.

5. The amoebae transferred from man to the rodent host have, in every case, presented no apparent morphological or racial change during the period of the experiment.

6. Four of the rats infected with *E. dysenteriae* and one infected with *E. coli* died of an apparent lung infection during the experiment. During the same period, three rats uninfected with human amoebae from the rat colony of one hundred and fifty animals died of the same lung infection. This fact indicates that rats infected with *E. dysenteriae* have a lowered resistance and are more susceptible to the lung infection than normal rats.

7. One of the four rats that died with *E. dysenteriae* showed an intestinal ulcer.

8. Infections of *E. dysenteriae* in rats and mice apparently assume a chronic form instead of an acute form as is reported in the case of kittens.

9. Both dead and normal cysts of human intestinal amoebae may be found for some hours after feeding in any part of the intestinal tract of experimental rats and mice. This indicates a difference in the viability of the cysts.

10. The findings of this investigation afford evidence in favor of the morphological constancy of species of parasitic amoebae, under conditions of change of host, and of the possibility of transfer of one species of parasitic amoebae from one species of host to another. Consequently heavy suspicion is thrown on the rat and mouse as possible carriers of the causative organisms of human amoebiasis.

Zoological Laboratory,
University of California.

Transmitted July 12, 1923.

E. LITERATURE CITED

Baetjer, W. A., and Sellards, A. W.

1914. The behavior of amoebic dysentery in lower animals and its bearing upon the interpretation of the clinical symptoms of the disease in man. Bull. Johns Hopkins Hosp., **25**, 237–241.

Brug, S. L.

1919. De entamoeben van de rat. Geneesk. Tijd. Nederl.-Indië, **59**, 1–15, pls. 1–2.

Chatton, E.

1917. L'éclosion des kystes et les premiers stages de l'évolution de l'amoebe dysentérique humaine chez le chat. Bull. Soc. Path. Exot., **10**, 834–841.

1918. L'amibiase intestinale expérimentale du cobaye à *Entamoeba dysenteriae*. Ses caractères speciaux: localization caecale, absence de dysenterie, importantes réactions hyperplasiques. Arch. Inst. Pasteur Tunis, **10**, 137–156.

Councilman, W. T., and Lafleur, H. A.

1891. Report in pathology. I. Amoebic dysentery. Johns Hopkins Hosp. Rept., **2**, 395–548, pls. 1–7.

Dale, H. H., and Dobell, C.

1917. Experiments on the therapeutics of amoebic dysentery. Jour. Pharm. and Exper. Therap., **10**, 399–459.

Dobell, C.

1919. The amoebae living in man (London, John Bale, Sous and Danielsson, Ltd.), vi + 155 pp., 5 pls.

Grassi, B.

1881. Intorno ad alcuni Protisti endoparassitici ed appartenati alle classi dei flagellati, lobosi, sporozoi, sciliati. Atti Soc. Ital. Sci. Nat., **24**, 135–224, pls. 1–4.

Huber.

1909. Untersuchungen über Amöebendysenteriae. Ztschr. f. klin. Med., **67**, 262.

Kartulis, S.

1891. Einiges über die Pathogenese der Dysenterieamöben. Centralbl. f. Bakt., **9**, 365–372.

Kessel, J. F.

1923*a*. Methods of obtaining amoeba-free rats for experimental infection with intestinal amoebae. Univ. Calif. Publ. Zool., **20**, 401–408.

1923*b*. The distinguishing characteristics of the parasitic amoebae of culture rats and mice. *Ibid.* (*In press.*)

Kofoid, C. A.

1923. Amoeba and man. Univ. Calif. Chronicle, **25**, 140–174 and 291–312, pls. 1–6.

Kofoid, C. A., and Swezy, O.

1921. On the free, encysted, and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine. Univ. Calif. Publ. Zool., **20**, 160–198, pls. 18–22, 3 figs. in text.

Kofoid, C. A., Kornhauser, S. I., and Swezy, O
1919. Criteria for distinguishing the *Endamoeba* of amoebiasis from other organisms. Arch. Int. Med., **24**, 35–50, 43 figs. in text.

Kruse, W., and Pasquale, A.
1894. Untersuchungen über Dysenterie und Leberabscess. Ztschr. f. Hyg., **16**, 1–148, pls. 1–6.

Lynch, K.
1915. The rat as a carrier of dysenteric amoeba. Jour. Am. Med. Assoc., **65**, 2232–2234.

Rudovsky, F.
1921. Über Rattenamöbiase. Wien. tierarzt. Monatssch., **8**, 1–9, pl. 7.

Wenyon, C. M.
1907. Observations on the Protozoa in the intestine of mice. Arch. f. Prot., Suppl. 1, 169–201, pls. 10–12.

Wenyon, C. M., and O'Connor, F. W.
1917. Human intestinal Protozoa in the Near East (London, Wellcome Bureau of Scientific Research), pp. 1–218, pls. 1–4.

Werner, H.
1908. Studien über pathogene Amöben. Arch. f. Schiffs- u. Tropenhyg., **12**, 419–436, pls. 1–6.

F. EXPLANATION OF PLATES

All figures are drawn from faecal smears fixed in hot Schaudinn's fluid and stained in iron haematoxylin. The magnification in all cases is 2500 diameters.

PLATE 38

Fig. 1. Mononucleate cyst of *Iodamoeba bütschlii* in human faecal material fed to rats.

Fig. 2. Mononucleate cyst of *Iodamoeba bütschlii* recovered in faecal material of rat.

Fig. 3. Motile *Endamoeba coli* with granular pseudopodium recovered from rat.

Fig. 4. Binucleate cyst of *Endolimax nana* in human faecal material fed to rats.

Fig. 5. Binucleate cyst of *Endolimax nana* recovered in faecal material of rat.

Fig. 6. Motile *Councilmania lafleuri*, with hyaline pseudopodia, recovered from rat.

Fig. 7. Mononucleate cyst of *Endamoeba dysenteriae* in human faecal material fed to rats.

Fig. 8. Mononucleate cyst of *Endamoeba dysenteriae* recovered in faecal material from rat.

Fig. 9. Four-nucleate cyst of *Endamoeba dysenteriae* in human faecal material fed to rats.

Fig. 10. Binucleate cyst of *Endamoeba dysenteriae* in human faecal material fed to rats.

Fig. 11. Binucleate cyst of *Endamoeba dysenteriae* recovered in faecal material from rat.

Fig. 12. Four-nucleate cyst of *Endamoeba dysenteriae* recovered in faecal material from rat.

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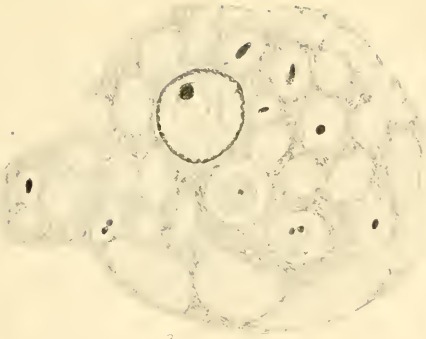
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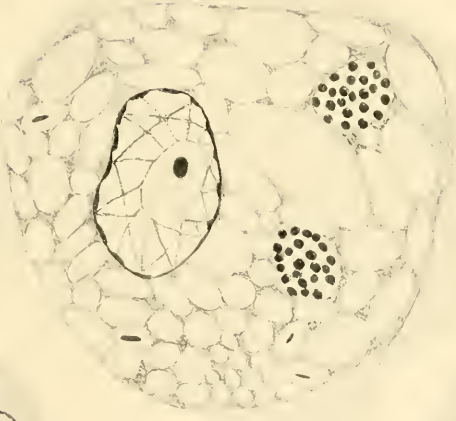
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PLATE 39

Fig. 13. Eight-nucleate cyst of *Endamoeba coli* in human material fed to rats.

Fig. 14. Eight-nucleate cyst of *Endamoeba coli* recovered in faecal material from rat.

Fig. 15. Eight-nucleate cyst of *Endamoeba coli* recovered in faecal material from rat.

Fig. 16. Eight-nucleate cyst of *Endamoeba coli* in human faecal material fed to rats.

Fig. 17. Binucleate cyst of *Councilmania lafleuri* recovered in faecal material from rat. Large glycogen vacuole, surrounded with chromatoidal bodies. Upper nucleus in telophase, showing chromosomes and karyosome having separated. Lower nucleus in prophase.

Fig. 18. Eight-nucleate cyst of *Endamoeba coli* recovered in faecal material from rat.

Fig. 19. Eight-nucleate cyst of *Councilmania lafleuri* in human faecal material fed to rats.

Fig. 20. Eight-nucleate cyst of *Councilmania lafleuri* recovered in faecal material from rat.

Fig. 21. Eight-nucleate cyst of *Councilmania lafleuri* in human faecal material fed to rats.

Fig. 22. Eight-nucleate cyst with bud of *Councilmania lafleuri* recovered in faecal material from rat. Note the somewhat massed type of karyosome which may be confused by some with the typical nucleus of *E. coli.*

Fig. 23. Eight-nucleate cyst of *Councilmania lafleuri* recovered in faecal material from rat.

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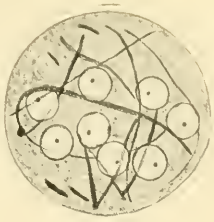

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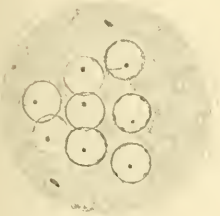

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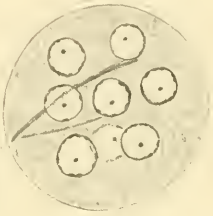

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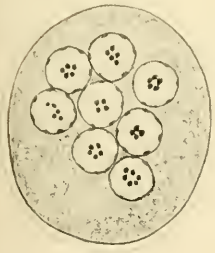

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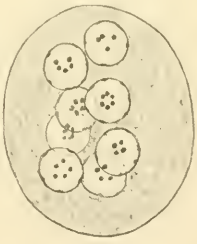

23

11. The Life Cycle of *Echinostoma revolutum* (Froelich), by John C. Johnson. Pp. 338-388, plates 19-25, 1 figure in text. May, 1920 ..... .60
12. On Some New Myriopods Collected in India in 1916 by C. A. Kofoid, by Ralph V. Chamberlin. Pp. 389-402, plates 26-28. August, 1920 ..... .20
13. Demonstration of the Function of the Neuromotor Apparatus in *Euplotes* by the Method of Microdissection, by Charles V. Taylor. Pp. 403-470, plates 29-33, 2 figures in text. October, 1920 ..... .85
Index, pp. 471–480.

Vol. 20. 1. Studies on the Parasites of the Termites. I. On *Streblomastix strix*, a Polymastigote Flagellate with a Linear Plasmodial Phase, by Charles Atwood Kofoid and Olive Swezy. Pp. 1-20, plates 1-2, 1 figure in text. July, 1919 .25
2. Studies on the Parasites of the Termites. II. On *Trichomitus termitidis*, a Polymastigote Flagellate with a Highly Developed Neuromotor System, by Charles Atwood Kofoid and Olive Swezy. Pp. 21-40, plates 3-4, 2 figures in text. July, 1919 ..... .25
3. Studies on the Parasites of the Termites. III. *On Trichonympha campanula* sp. nov., by Charles Atwood Kofoid and Olive Swezy. Pp. 41-98, plates 5-12, 4 figures in text. July, 1919 ..... .75
4. Studies on the Parasites of the Termites. IV. On *Leidyopsis sphaerica* gen. nov., sp. nov., by Charles Atwood Kofoid and Olive Swezy. Pp. 99-116, plates 13-14, 1 figure in text. July, 1919 ..... .25
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7. On the Free, Encysted, and Budding Stages of *Councilmania lafleuri*, a Parasitic Amoeba of the Human Intestine, by Charles Atwood Kofoid and Olive Swezy. Pp. 169-198, plates 18-22, 3 figures in text. June, 1921 ..... .60
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9. The Micro-Injection of *Paramaecium*, by Chas. Wm. Rees. Pp. 235-242. April, 1922 ..... .20
10. On *Balantidium coli* (Malmsten) and *Balantidium suis* (sp. nov.), with an account of their neuromotor apparatus, by J. Daley McDonald. Pp. 243-300, plates 27, 28, 15 figures in text. May, 1922 ..... 1.00
11. Mitosis in *Endamoeba dysenteriae* in the Bone Marrow in *Arthritis deformans*, by Charles Atwood Kofoid and Olive Swezy. Pp. 301-307, 7 figures in text.
12. *Endamoeba dysenteriae* in the Lymph Glands of Man in Hodgkin's Disease, by Charles A. Kofoid, Luther M. Boyers, M.D., and Olive Swezy. Pp. 309-312, 4 figures in text.
Nos. 11 and 12 in one cover. April, 1922 ..... .25
13. Mitosis in the Encysted Stages of *Endamoeba coli* (Loesch), by Olive Swezy. Pp. 313-332, plates 29-31. May, 1922 ..... .30
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ON THE GENUS COUNCILMANIA, BUDDING INTESTINAL AMOEBAE PARASITIC IN MAN AND RODENTS

BY
CHARLES A. KOFOID, OLIVE SWEZY AND JOHN F. KESSEL

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CONTENTS

PAGE

Introduction 431
Councilmania Kofoid and Swezy 1921 432
Nomenclature 433
Councilmania lafleuri Kofoid and Swezy 1921 435
Councilmania muris (Grassi) 438
Councilmania decumani (Rudovsky) Kessel 439
Key to species of *Councilmania* 443
Summary 444
Literature cited 445

INTRODUCTION

The parasitic intestinal Protozoa of the mammals, including man, reveal many suggestions of an evolutionary origin from stocks represented in comparable parasites of the lower vertebrates. This relationship is exhibited by the fact that every protozoan genus of this faunal assemblage of the intestine of man is represented either by other species in the lower vertebrates or by closely related or more primitive genera. These genera may even extend to invertebrate hosts; for example, *Endamoeba* is found not only in mammals but also in the cockroach, while *Craigia* (*Paramoeba*) occurs also in *Sagitta*. The trichomonad flagellates have an as yet little known, but certainly wide distribution in both vertebrates and invertebrates. The most complex derivative of this group, the genus *Giardia*, has

not been found below amphibians in the vertebrate scale, and *Pentatrichomonas*, the most complex of the trichomonads proper of mammals, has thus far been found only in man.

The discovery by Kofoid and Swezy (1921*a*, *b*) of a type of amoeba in man generically distinct from those previously described from the vertebrates, naturally raises the question as to whether or not species of this genus occur in other mammals.

As a result of the investigations of Kessel (1923*a*, *b*, *c*) as University Fellow, 1922–1923, in our laboratory, two additional species of *Councilmania* have been distinguished in culture mice and rats of our laboratory colony. Experimental rearing of these species by transfers to amoeba-free hosts and the like transfer and successful culture of the human parasitic Protozoa, including *Endamoeba coli* and *Councilmania lafleuri* (see Kessel, 1923*b*), have enabled us to verify, supplement, and extend our observations on this genus. It is the purpose of this paper to present a brief summary of these results and relate them to the findings of Kofoid and Swezy (1921*a*, *b*) and to their subsequent work on *Councilmania* shortly to be published in fuller detail.

Acknowledgments are made to the Research Board of the University of California, to the Carnegie Institution, and to friends of the University contributing to the support of this work, for funds which have made the investigation possible. Much of our material has been obtained in routine examinations made in the Division of Parasitology of the California State Board of Health, in part by Miss Inez Smith.

Councilmania Kofoid and Swezy 1921

This genus is distinguished by a group of characteristics which no other parasitic amoeba, or group of parasitic amoebae presents. These are: (1) the karyosome is centrally located (in the resting phase of the nucleus in the cyst), dispersed in numerous subequal, discrete granules; (2) the nuclear membrane in cysts is generally faint, and not often heavily or continuously encrusted with peripheral chromatin; (3) the cysts bud in the caecum or colon of the host by the escape of uninucleate amoebulae from a definite pore; and (4) the pseudopodia are ectoplasmic, broad, rounded, very hyaline, are formed very suddenly, and may persist as clear lobes for extended periods of time. The three known species are all parasitic. The active stages are found in the caecum and colon and the cysts also occur (in the rodent hosts) in the caecum. The cysts,

either with or without budding, occur in the stools of the host, though not regularly. In rodent hosts they are much more sporadic than in the human one.

The type species is *Councilmania lafleuri* Kofoid and Swezy (1921). Other species may be expected to be found in the wild rodents.

This species prior to 1921 was confused with *Endamoeba coli* (Loesch) Schaudinn, and since its publication has been included in the synonymy of *E. coli* by Wenyon (1922), Brumpt (1923), and others, we believe incorrectly and without adequate, critical reëxamination of material obtained under critical conditions.

NOMENCLATURE

Amoeba muris was originally described by Grassi (1881) from rats and mice, but only in the motile stages. His account affords no basis for distinguishing between the two forms Kessel (1923*a*) has found in rats and mice. However, Wenyon's (1907) figures and account of the cysts of the amoeba which he designates as *Amoeba muris* are sufficiently detailed to afford a basis for the specific recognition of one of the two species. He described but one species in the mouse and gives no account of the amoebae of the rat. His account apparently applies wholly, or in the main, to one species, but in his plates there are at least three figures of cysts (his pl. 10, figs. 20, 33, 34) which clearly differ in nuclear structure from the others delineated by him. He makes no reference to these differences in his text or in his diagram of *Amoeba muris*. His references to the figures do not deal with any of the distinctive features of these three cysts, namely, the peripheral chromatin blobs which characterize the nucleus of *Councilmania decumani* (Rudovsky).

We conclude, therefore, that Wenyon's paper (1907) deals primarily with the species *muris* and thus emends Grassi's account and sufficiently establishes its characteristics, to wit, budding in the cysts and the absence of large chromatin blobs of peripheral chromatin on the nuclear membrane in the cysts. The budding process, the often faint nuclear membrane, and the diffused karyosome which characterize *Councilmania* are recognizable in his figures, though not always portrayed with accuracy of detail, especially the numerous prophases of mitosis. We believe that three of his figures (his pl. 10,

figs. 20, 33, 34) are of *Councilmania decumani.* These have coarse blobs of peripheral chromatin on the nuclear membrane, and the nuclei are somewhat smaller than the general run in *C. muris.* It appears from this that Wenyon (1907) found in mice the two species which we have found, but recognized only one of them, *C. muris.*

Brug (1919), working on the amoebic infections of the wild *Mus rattus* of Java, encountered two amoebae (possibly three). To one of these he gave the name *Entamoeba muris,* the other he called *Entamoeba tetragena.* This last he found in a natural infection in a wild rat and succeeded in one instance in inducing an infection in the rat by feeding cysts from human stools. His figures and discussion convince us that he was dealing in these instances with an infection by *Endamoeba dysenteriae* (Councilman and Lafleur).

His figures (his pl. 1) of the amoeba called by him *Entamoeba muris* clearly portray an amoeba with a few coarse blobs of peripheral chromatin on the nuclear membrane in the encysted stages in stained material. Two of his figures (17 and 18) are indeterminate and one only (fig. 15) may possibly belong to *Councilmania muris.* The others are clearly referable, not to *C. muris,* but to *C. decumani* Rudovsky (1921).

In 1921 Rudovsky failed to find *Endamoeba muris* (Grassi) Wenyon in mice but described an amoeba which he found in 139 wild rats as *Entamoeba muris decumani.* This amoeba is correctly characterized as having thin nuclear membrane, small karyosome, and a small amount of chromatin. He noted the difficulty in getting a typical glycogen reaction of the contents of the vacuole in the binucleate stage when the glycogen vacuole has its greatest volume. He figures 2-, 4-, 8-nucleate cysts, not very clearly, but with sufficient detail to be recognizably distinct from Wenyon's (1907) main type which he (Rudovsky) cites as *E. muris.* He does not figure, and apparently did not find the species *muris.* We conclude that this species of Rudovsky (1921) is identical with one we find in both rats and mice and that it is different from the one which Wenyon regarded as Grassi's *Amoeba muris.*

Wenyon (1907) seemingly found both species (but recognized only one) in the mouse. Brug (1919) seemingly found both in the rat (but recognized only one) and applied Grassi's name to it although it differed from the species to which Wenyon applied the name. Rudovsky (1921) found only one species in the rat but recognized its differences from the species *muris.*

We have found both species in both rats and mice of our laboratory colonies, and refer them to the genus *Councilmania* because both bud in the encysted stage, and have distributed karyosomes, at least at some stages.

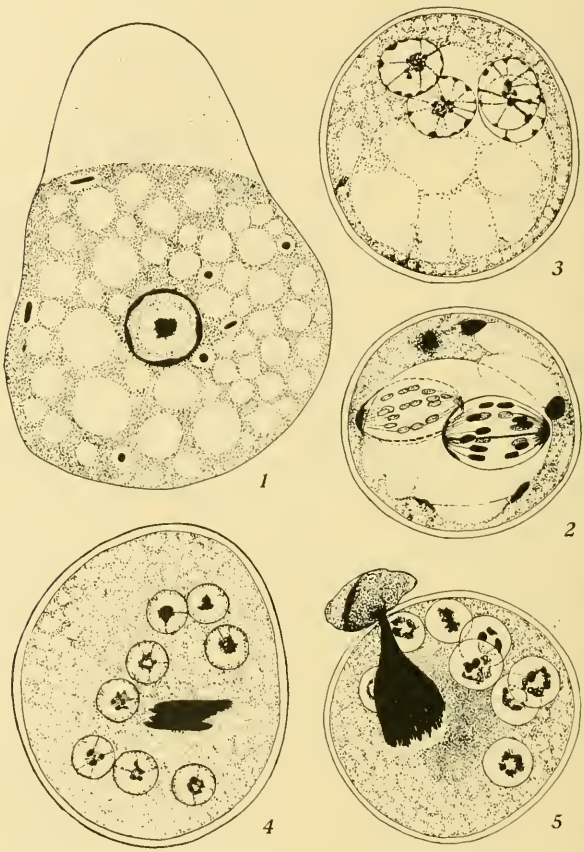
The following summary of the characteristics of the three known species belonging to the genus *Councilmania* will facilitate their determination.

Councilmania lafleuri Kofoid and Swezy 1921

The motile amoeba is characterized by explosive thrusting out of broadly rounded ectoplasmic pseudopodia, one, often two, less frequently three at one time. In life these are absolutely clear and hyaline and may linger unchanged for some time before the endoplasm moves in the same direction. They are often shorter and broader than shown in figure 1. The line of separation between endoplasm and ectoplasm is sharply marked. The nucleus has in this phase a well developed film of peripheral chromatin, which may retract in scattered blobs, five to eight in optical section. Inside of this is a zone traversed by granular radii, the outer part of which is more granular and the inner forms a clearer halo about the *centrally located* karyosome. The karyosome is not solid and spherical but has irregular margins indicating that it is an assemblage of granules. These are more closely packed together in the motile stage than in the cysts. The endoplasm contains food vacuoles with bacteria, the cysts of other protists, and, at times, red blood corpuscles.

The cysts occur in the 1-, 2-, 4-, and 8-nucleate stages (figs. 2–5) in the stools, but the earlier phases are best found in the liquid stool after a saline purge. The glycogen vacuole is best seen in the binucleate cyst (fig. 2) when it crowds the nuclei to the periphery. It may be single or multiple and may resist prolonged iodine staining at times. It stains in sections in Best's carmine. As it disappears, the chromatoidal bodies (fig. 2) form as flakes and splinters about its periphery and in later phases concentrate in a centrally located bundle (figs. 4, 5) with ragged or irregular ends.

The nuclei of the earlier encysted stages often appear in premitotic phases. In some of these (fig. 2) eight chromosomes and a meridional intradesmose can be demonstrated. The most typical nuclei in the resting phases can be best seen in the 8-nucleate cysts (figs. 4, 5). These are characterized by a slight amount of peripheral chromatin (fig. 4), or none at all (fig. 5), and the intermediate zone is often very clear so that the nuclei stand out in the cytoplasm as



Figs. 1–5. *Councilmania lafleuri* Kofoid and Swezy (1921). × 2500. From camera lucida drawings of amoebae from smears of human faeces fixed in hot Schaudinn's fluid and stained in iron haematoxylin.

Fig. 1. Motile amoeba showing hyaline pseudopodium, sharp demarcation of ectoplasm and endoplasm and nucleus with central, massed karyosome, with ragged outline indicating massing of granules; clear zone, spoke radii, and peripheral chromatin.

Fig. 2. Binucleate cyst with subcentral empty glycogen vacuole, with adjacent accessory vacuoles and flakes of chromatoidal substance developing on the periphery of the vacuoles. The two nuclei are in the early anaphase with polar centrosomes, joined by a meridional intradesmose. Eight pairs of chromosomes are in transit toward the poles of the spindle.

clear spheres. The karyosome is large, central, and composed of discrete granules, sometimes densely massed, sometimes dispersed in a granular sphere or in a more or less complete ring. This dispersal takes on a variety of aspects and is modified by the approach of mitosis when the karyosome migrates to the nuclear membrane and divides. The two moieties migrate to the poles of the spindle and spin out the meridional intradesmose between them. Except during this period of migration and mitosis the karyosome tends to assume a central location in the nucleus rather than an excentric one. The excentricity, if present, is rarely marked. The nuclei of the 2-, 4-, and 8-nucleate cysts have an average and range of 6 microns, 5–6 microns, 5.3 microns, 4–5.6 microns, 3.5 microns, 3–4 microns respectively in diameter. The cysts are spheroidal, or ellipsoidal, often the latter, with the longest diameter not over 1.1 the shortest one. The cysts are generally 16 to 20 microns in longest diameter and range from 8 to 39 microns. The cysts reach an 8-nucleate phase in the bowel, and rarely go to a 16-nucleate condition before the budding process begins. This is brought about by the opening of a minute circular pore through the cyst membrane at or near the end of the centrally located chromatoidal mass whose substance is dissolved in the cytoplasm which escapes through the pore and causes it to stain more deeply. The nuclei slip out with the cytoplasm and amoebulae, each with a single nucleus, successively detach themselves from the cyst. Mitosis may proceed within the cyst during the process.

Small amoebulae occur in stained smears adjacent to budding cysts and empty cysts may sometimes be found in the stool. Chromophile strands or ridges in the peripheral cytoplasm (figs. 6, 7) in a single or a tripartite arc are formed prior to the opening of the pore and appear to be associated with the chromatoidal body and the subsequent location of the pore. The habitat is presumably the colon and caecum. Cysts have been found by us in the contents of the duodenal bucket by the Lyon method. In artificially infected culture rats this species occurs in the caecum.

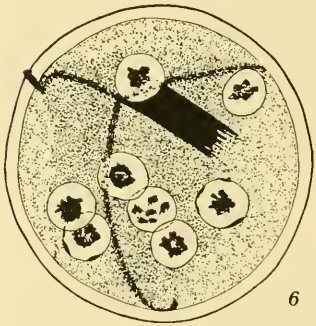
Fig. 3. Four-nucleate cyst with disappearing glycogen vacuole, traces of chromatoidal substance, four nuclei with dispersed granular, centrally or subcentrally located karyosomes, and considerable peripheral chromatin.

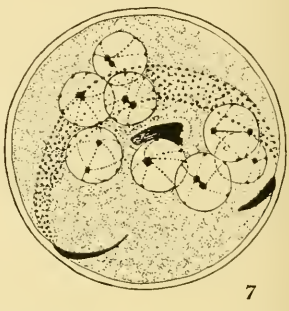
Fig. 4. Eight-nucleate cyst with nuclei with less peripheral chromatin and massed ring-shaped, central karyosomes, and centrally located chromatoidal body with ragged ends.

Fig. 5. Budding cyst with eight nuclei, chromatoidal substance contributing to the outpouring cytoplasm, pore at the end of the chromatoidal body. Karyosomes ring-shaped and centrally located, nuclear membrane with little peripheral chromatin.

Councilmania muris (Grassi)

The motile forms of the amoebae belonging to the genus *Councilmania* found in rats and mice cannot easily be distinguished in fresh smears from the motile forms of *C. lafleuri* except by size. The average size of rounded motile *C. decumani* and *C. muris* is 19 microns while *C. lafleuri* may attain a size of 63 by 35 microns. The pseudopodia are, without exception, hyaline in structure. This fact is borne out by all the previous investigations of the amoebae of rats and





Figs. 6 and 7. *Councilmania lafleuri* Kofoid and Swezy 1921. × 2500. From camera lucida drawings of amoebae from smears of human faeces fixed in hot Schaudinn's fluid and stained in iron haematoxylin.

Fig. 6. Initial stage of emergence of bud. Tripartite chromophile strands below with the pore at the tip of the upper one. The deeply stained cytoplasm is beginning to emerge from the pore. The nuclei have centrally located, massed or ring-shaped karyosomes.

Fig. 7. Eight-nucleate cyst with broad, rather diffuse chromophile strand or ridge (below). Note the depression at the surface. It is not a wrinkle of the cyst wall but a cytoplasmic differentiation. The chromatoidal body is vertical, is greatly reduced in size, and is enveloped in a clear zone. Its lower end points toward the middle of the chromophile ridge. The nuclei are in early prophases with small karyosomes on or near the membrane. The karyosomes are in most instances divided and the intradesmose is in process of formation. When not divided the karyosome at this stage of mitosis is usually peripheral in location.

mice, namely, by Grassi (1881), Wenyon (1907), Brug (1919), and Rudovsky (1921). For a detailed description of the amoeboid movement of the amoebae, see Kessel (1923*c*).

The normal habitat of this amoeba is the caecum, where it feeds upon bacteria, yeasts, and on other Protozoa. So far as known, it is non-pathogenic to the rat.

In specimens stained with iron haematoxylin the nuclear structure of *C. muris* is distinctive. The nuclear membrane is very thin and is seldom, if ever, encrusted with chromatin granules. The karyosome is characterized by the mass of 5 to 8 dispersed granules, arranged centrally or subcentrally. This diffuse condition of the karyosome is characteristic of every cyst in a resting phase encountered in this species (fig. 12).

A glycogen mass is commonly found in the 1-, 2-, and 4-nucleate cysts (figs. 15–17). In these stages, phases of mitosis are usually present. Mitosis is similar in this species to the process in other species of parasitic amoebae in that the nuclear membrane remains intact during division and that an intradesmose connects the centrosomes of the spindle. The number of chromosomes found in *C. muris* is six (fig. 11).

The 8-nucleate cyst is the typical or characteristic cyst of this species. The nuclei in the resting stage are characterized by a very thin nuclear membrane, often very difficult to find, its position being determined by the limits of the cytoplasm surrounding the nucleus. The karyosome retains the typical dispersed condition found in the nucleus of the motile forms.

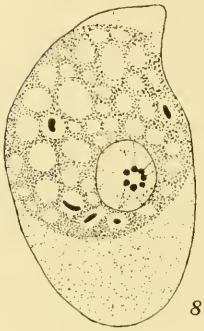
The average diameter of the nuclei in the two-nucleate cysts of this species is 5.5 microns, the range being 5 to 6 microns; of the 4-nucleate cysts the average diameter of the nuclei is 4.8 microns, the range being 4 to 5.2 microns; while in the 8-nucleate cysts the average diameter of the nuclei is 3.4 microns, the range being from 2.75 to 3.75 microns.

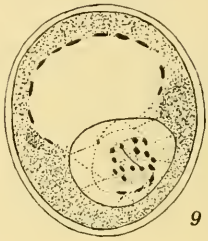
Chromophile ridges are commonly present in the 8-nucleate cysts in this species (fig. 12). The chromatoidal bodies are typically bundles of splinters with jagged ends (figs. 10, 13). In the 8-nucleate cyst the point of the chromatoidal body is generally found in the region of the pore. Budding occurs in this species in a manner similar to the type of budding found in *C. lafleuri* (fig. 13).

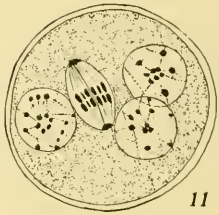
The cysts vary from 14 microns to 19 microns in diameter, with an average of 15.8 microns. It occurs in both mice and rats of our culture colony.

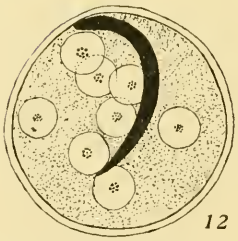
Councilmania decumani (Rudovsky) Kessel

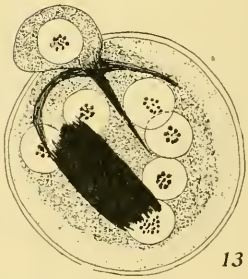
The motile forms of *C. decumani* are very similar to the motile forms of *C. muris* in that the vacuolation and food materials are identical and the pseudopodia are broadly rounded and hyaline in structure. There is also no marked difference in size.











Figs. 8–13. *Councilmania muris* (Grassi, 1881) Kessel, emend. 1923. × 2500. Camera lucida drawings of amoebae from smears of rat faeces fixed in hot Schaudinn's fluid and stained in iron haematoxylin.

Fig. 8. Motile amoeba showing hyaline pseudopodium and posterior tooth-like projection. Note the marked differentiation between endoplasm and ectoplasm. Nucleus showing karyosome of dispersed chromatin granules, and thin nuclear membrane with no encrusted chromatin.

Fig. 9. Mononucleate cyst, showing glycogen mass surrounded by small chromatoidal bodies. Nucleus with thin membrane and dispersed granules in a darkly staining region. Two of the granules are connected by an intradesmose.

Fig. 10. Binucleate cyst showing glycogen mass in center and chromatoidal body at edge. Nuclei in anaphase, showing spindle and six chromosomes.

The nuclear structure of the motile stages is, however, quite different in the individuals stained with iron haematoxylin where the karyosome, instead of appearing as a number of dispersed granules, occurs as a sphere or a crescent (fig. 15). The nuclear membrane is slightly heavier than the nuclear membrane of *C. muris* and is commonly encrusted with discrete chromatin granules (fig. 15).

This massing of chromatin material in blobs on the nuclear membrane in the cysts is a characteristic of this species, such a condition being common in some stages of nuclear division, and in the resting stage, but not at the metaphase (fig. 17). The karyosome of this species is more massed than the karyosome of *C. muris* or *C. lafleuri*, but shows a tendency to dispersion throughout by minute irregularities on its margins. The karyosome is never so small nor so characteristically massed as the karyosome of *Endamoeba coli* or *E. dysenteriae*.

The stages of mitosis are similar to those of other parasitic amoebae (fig. 17). Four chromosomes, including one minute one, are characteristic for this species.

The typical resting nucleus is seen in figure 18. It is characterized by a thin but distinct nuclear membrane upon which several heavy chromatin blobs are encrusted. The number may vary from two to six or eight, but usually four chromatin blobs are present. The karyosome is excentric, larger than the karyosome of *Endamoeba coli*, and shows a tendency to dispersion.

The nuclei of *Councilmania decumani* are on the whole smaller than the nuclei of *C. muris*. Their average diameter in the binucleate cysts is 4.6 microns and the range 4 to 5.2 microns; while the average diameter in the 4-nucleate cysts is 3.8 microns, the range being from 3.1 to 4.4 microns, and the average diameter in the 8-nucleate cysts is 2.8 microns, the range being from 2.5 to 3 microns.

This species of amoeba is poorer in chromatoidal material than *C. muris*, the presence of masses of chromatoidal splinters being the

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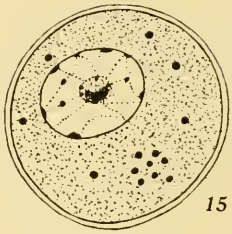
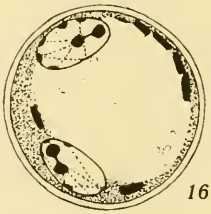
Centrosomes are connected by an intradesmose. The nuclear membrane is thin with no encrusted chromatin.

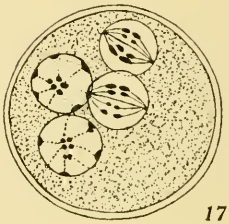
Fig. 11. Four-nucleate cyst, one nucleus in metaphase and the other three in prophase. Note dispersed condition of chromatin granules of nuclei in prophase, The membranes are characteristically thin with no encrusted chromatin.

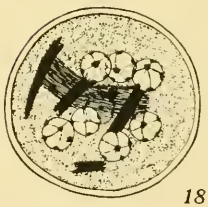
Fig. 12. Eight-nucleate cyst showing typical chromophile ridge. The nuclei show the characteristic, central, dispersed karyosome, and thin nuclear membrane with no encrusted chromatin.

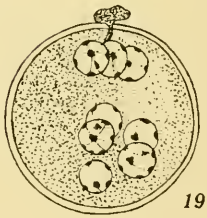
Fig. 13. Budding cyst, showing tripartite chromophile ridge and exceptionally large chromatoidal mass with splintered ends. The karyosomes are dispersed granules and the nuclear membranes are faint.











Figs. 14–19. *Councilmania decumani* (Rudovsky, 1921) Kessel, emend. 1923. × 2500. Preparations same as in figures 8–13.

Fig. 14. Motile amoeba showing hyaline, rounded pseudopodia with sharp demarcation between ectoplasm and endoplasm; nucleus with somewhat massed crescentric karyosome in darkened halo and chromatin granules encrusted on nuclear membrane.

Fig. 15. Uninucleate cyst with no glycogen mass but with a number of small, spherical chromatoidal bodies. The karyosome is located in a darkly staining halo containing four small granules. Nuclear membrane is encrusted with heavy chromatin blobs.

Fig. 16. Binucleate cyst with glycogen mass on the margin of which are chip-shaped chromatoidal masses. Nuclei in early prophase, the karyosome breaking into two portions connected by an intradesmose. Characteristic peripheral chromatin encrusted on nuclear membrane.

exception rather than the rule. Small masses with irregular ends (fig. 19) or chips or spheres of chromatoidal substance (fig. 15, 16) are commonly encountered.

The budding cyst of *C. decumani* is characterized by the absence of chromatoidal material and by few chromophile ridges, only one small ridge having been found in this species to date. Buds, however, are common and are produced by the same method of reproduction in this species as in the others belonging to this genus.

The normal cysts range from 12 to 20 microns in diameter but one large 16-nucleate cyst has been found, measuring 22 microns. The average diameter of the cysts of this species is 15.5 microns. This species occurs in both mice and rats of our culture colony.

KEY TO SPECIES OF *COUNCILMANIA*

Cysts spheroidal to ellipsoidal, 8–34, generally 16–20 microns in longest diameter; in 8-nucleate cysts the nuclear membrane is generally very faint with little peripheral chromatin; diameter of nuclei 3.5 microns; karyosome central, composed of numerous coarse, irregular granules; 8 chromosomes ..... *lafleuri*

Cysts generally spherical, 15.8 (13–19) microns in diameter; very little peripheral chromatin at any stage in the nuclei of the cysts; nuclear membrane very faint; diameter 3.4 (2.75–3.75) microns; karyosome central composed of 5–7 dispersed fine, uniform granules; 6 chromosomes ..... *muris*

Cysts spherical, 15.5 (12–22) microns in diameter; peripheral chromatin in several rather large, distinct blobs; nuclear membrane distinct, diameter 2.8 (2.5–3.0) microns; karyosome excentric, composed of massed granules, 4 chromosomes .... *decumani*

Fig. 17. Four-nucleate cyst, two nuclei in prophase, showing karyosome breaking up and peripheral chromatin blobs. The other two nuclei are in early anaphase showing the spindle, the four chromosomes having just divided. The centrosomes are connected by an intradesmose.

Fig. 18. Eight-nucleate cyst showing clustered chromatoidal bodies. The nuclei are characteristic resting nuclei of this species with excentric karyosome more massed than in *C. muris* but with a tendency to dispersion and with characteristic chromatin blobs on nuclear membrane.

Fig. 19. Eight-nucleate cyst with no chromatoidal bodies and with bud in early formation. Note absence of chromophile ridge. The nuclei show the typical structure common to this species.

SUMMARY

The genus *Councilmania* is characterized by clear, hyaline pseudopodia, a nuclear membrane usually not heavily encrusted with peripheral chromatin, a karyosome that in the resting phase in the cyst is composed of numerous discrete granules, and by a budding process in which uninucleate amoebulae escape from the cyst in the caecum or colon of the host by a definite pore. The pore is formed near the end of the chromatoidal mass and on or near the chromophile ridge.

The type species is *C. lafleuri* found in the human intestine. In this species little peripheral chromatin is found on the nuclear membrane, and the karyosome is composed of numerous coarse granules. Eight chromosomes are found at mitosis.

C. muris is found in rats and mice. The karyosome is central and composed of 5 to 7 dispersed, fine, uniform granules. Six chromosomes are present during mitosis. No chromatin blobs occur on the nuclear membrane.

C. decumani is also found in rats and mice. The nuclear membrane is clearly marked with several rather large, distinct blobs, and a karyosome composed of massed granules. Four chromosomes are found at mitosis.

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Transmitted July 23, 1923.

LITERATURE CITED

Brug, S. L.

1919. De entamoeben van de rat. Genees. Tijdschr. Nederlandsch-Indië, **59**, 1–16, pls. 1–2.

Brumpt, E.

1922. Précis de parasitologie (Paris, Masson), ed. 3, xv + 1216 pp., 5 pls., 736 figs. in text.

Grassi, B.

1881. Intorno ad alcuni protisti endoparassitici ed appartenenti alle classi dei flagellati, lobosi, sporozoi e ciliati. Atti Soc. Sci. Nat., **24**, 135–224, pls. 1–4.

Kessel, J. F.

1923*a*. The distinguishing characteristics of the intestinal amoebae of culture rats and mice. Univ. Calif. Publ. Zool. (*In press.*)

1923*b*. Experimental infection of rats and mice with the common intestinal amoebae of man. *Ibid.*, **20**, 409–430, pls. 38–39.

1923*c*. Methods of securing amoeba-free rats for experimental infection with intestinal amoebae. *Ibid.*, **20**, 401–408.

Kofoid, C. A., and Swezy, O.

1921*a*. *Councilmania lafleuri*, a new amoeba of the human intestine. Proc. Soc. Exper. Biol. Med., **18**, 310–312.

1921*b*. On the free, encysted, and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine. Univ. Calif. Publ. Zool., **20**, 170–189, pls. 18–22, 3 figs. in text.

Rudovsky, F.

1921. Ueber Rattenamoebiase. Weiner tierärz. Monatsschr., **8**, 636–646, pl. 7.

Wenyon, C. M.

1907. Observation on the Protozoa in the intestine of mice. Arch. Prot., Suppl. 1, pp. 169–201, pl. 10–12, 1 fig. in text.

1922. On three supposed new *Entamoeba* of Man. Trop. Dis. Bull., **19**, 19–22.

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10. A New Morphological Interpretation of the Structure of *Noctiluca*, and Its Bearing on the Status of the Cystoflagellata (Haeckel), by Charles A. Kofoid. Pp. 317-334, plate 18, 2 figures in text. February, 1920	.25

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MORPHOLOGY AND BINARY FISSION OF MENOIDIUM INCURVUM (FRES.) KLEBS

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CONTENTS

	PAGE
Introductory and historical	448
Material and technique	449
General morphology	450
Neuromotor system	451
Gullet and reservoir	452
The nucleus	453
Habits and activities	453
Nuclear division	454
Resting stage	454
Prophase	455
Metaphase	455
Anaphase	456
Telophase	456
Binary fission	457
Paradesmose	458
Gullet and reservoir	459
Discussion	460
The resting nucleus	462
The endosome	463
Prophase	465
Metaphase	466
Anaphase and telophase	467
Summary	468
Literature cited	469
Explanation of plates	474

INTRODUCTORY AND HISTORICAL

Early in the spring of 1921 there appeared in great abundance, in one of my aquaria, a small euglenoid flagellate, which has been identified as *Menoidium incurvum* (Fres.) Klebs. This form was first described by Fresenius (1858) as *Rhabdomonas incurva.* Stein (1878) later found this species but considered it to be merely a "jugendliche Form" of his *Astasia proteus* (= *Distigma proteus* Ehrb.). Seligo (1885) found, in the same culture, *Rhabdomonas incurva, Astasiopsis distorta* Dnj., and a so-called intermediate form. Influenced by the earlier statement of Stein he concluded that, since he found no division stages of his *Rhabdomonas,* this flagellate was a stage in the life history of *Astasiopsis.* However, both Bütschli (1883–1889) and Klebs (1883) later recognized *Rhabdomonas incurva* as a distinct species; finally, Klebs (1892), on the basis of the resemblance between *Menoidium pellucidum* Perty and *Rhabdomonas incurva* Fresenius, included the latter in the genus *Menoidium* Perty:

> Ich möchte jetzt die Gattung weiter fassen als Perty, Stein und ich selbst es früher gethan haben, indem ich die *Rhabdomonas incurva* hinzuziehe, da in der That der Unterschied zwischen dieser Art und *Menoidium pellucidum* viel geringer ist als derjenige zwischen einzelnen *Euglena*-resp. *Phacus*-Arten. Die Gattung *Menoidium* umschliesst dann die starren, eingeisseligen, *Astasia*-ähnlichen Flagellaten.

The species *Menoidium incurvum* is described by Klebs (1892) as follows:

> Körper cylindrisch, an beiden Enden abgerundet, meist etwas gekrümmt. Plasmamembran mit weit von einander stehenden Längsstreifen versehen. Länge, 16–21μ; Breite, 7–8μ.

Klebs's description coincides with my own observations, with the addition that these flagellates always contain a number of nearly colorless paramylum bodies, clumped together usually at the anterior end, although sometimes present at both ends (pl. 40, figs. 1 and 6). Since nuclear division and binary fission have not heretofore been described in this flagellate, I shall attempt to discuss these processes in this paper.

I am indebted to Dr. Robert C. Rhodes of Emory University, at whose suggestion this investigation was undertaken, for his advice and assistance; also, I wish to thank Professor Charles A. Kofoid, in whose laboratory this work was completed, for his invaluable criticisms and suggestions.

MATERIAL AND TECHNIQUE

Abundant material was obtained from an aquarium at Emory University, Atlanta, Georgia. The flagellates became dominant early in March, 1921, and were still plentiful in July. The aquarium in which the form occurred measured about 36 by 18 by 24 inches, and was filled to a depth of five or six inches with a mixture of mud, sand, sawdust, old leaves, and straw. A small turtle and several large tadpoles had been placed in the aquarium several weeks before the appearance of the flagellates, and were kept there continuously. *Menoidium* appeared to be uniformly distributed throughout the aquarium, there being no marked accumulation at the surface.

Several new cultures have been started at the University of California from samples of the original material sent me from Emory University. *Menoidium* has been present in these cultures fairly constantly for the past year and a half, although never in such abundance as in the first aquarium. Various attempts have been made to obtain pure cultures in peptone and beef extract solutions, boiled hay infusions, and different combinations of these, but none was markedly successful. Some of the Protozoa which have occurred in the cultures in association with *Menoidium* are: *Euglena*, *Petalomonas*, *Cryptoglena*, *Amoeba*, *Difflugia*, *Arcella*, *Chilomonas*, *Coleps*, *Stylonychia*, *Euplotes*, *Loxophyllum*, *Centropyxis*, *Cyclidium*, and *Colpidium*. In addition several species of small Crustacea, rotifers, diatoms, and green algae have usually been present.

In fixation, both the cover-glass flotation and the centrifuge methods have been used to accumulate material in quantity; the best results have been obtained by centrifuging. As fixing agents, strong and weak solutions of Flemming's fluid, and Schaudinn's fluid (both hot and cold solutions) have been used; Schaudinn's sublimate-alcohol has been more satisfactory. All staining has been done by the cover-glass method. In the flotation method as well as with centrifuged material, the cover-slips were smeared with a thin film of Mayer's egg albumen fixative; in the case of material killed after centrifuging, the organisms were pipetted onto the smeared covers and then stained in the usual way. Among the stains tried in addition to alcoholic and aqueous iron-alum haematoxylins are: Delafield's haematoxylin, the mixtures of Mallory and Mann, Benda's safranin light-green,

polychrome blue and orcein, Prenant's triple; and as counterstains after haematoxylin: eosin, erythrosin, light green, methyl green, methylene blue, Congo red, Bordeaux red, orange G, and safranin. As nuclear stains, none has been as satisfactory as the aqueous and alcoholic iron-alum haematoxylins; of the counterstains, Bordeaux red, eosin, methylene blue, orange G, and light green have produced good results.

As special stains for rhizoplasts and basal granules of flagella, a weak solution (0.1 per cent) of Bordeaux red, as recommended by Rhodes (1919), and the solution of Gicklhorn (1921) have been used before staining in iron-haematoxylin. The organisms were stained for twelve to thirty-six hours in either of these solutions and then transferred directly to iron-alum. Both stains are good, but the use of Bordeaux red has been especially advantageous.

GENERAL MORPHOLOGY

Menoidium incurvum is characterized by a rigid, elongate bean-shaped body (pl. 40, fig. 1), ranging in length from 15 to 25μ. Even under a 4 mm. objective the surface of the living flagellate shows regularly spaced longitudinal striations which are some distance apart (pl. 40, figs. 1 and 6). As determined by a study of optical cross-sections in stained material, the number of these varies from ten to fifteen for flagellates whose nuclei are in the prophase or resting stage; the greater number of those examined possess fourteen striations. The number of these striations is correlated with the circumference of the organisms; as the flagellate enlarges with the approach of binary fission, the number is seen to be increased (pl. 40, fig. 2). Posteriorly the striations converge to a point (pl. 40, fig. 3); anteriorly they extend to the margin of the gullet (pl. 40, fig. 4). In optical cross-sections (pl. 40, fig. 5) their thickness is approximately the same as that of the region between them, so that they probably do not represent appreciably thickened parts of the pellicle.

The endoplasm contains a variable number of plastids (pl. 40, figs. 1 and 6), ranging from practically colorless to a very light green; these were not stained in any of the stains used and are probably paramylum bodies, since they appear tinged with yellow when treated with solutions of iodin. In the living flagellates these plastids

are clumped together at either one or both ends of the animal (pl. 40, figs. 1 and 6), usually only at the anterior end and never altogether at the posterior end; in no case have they been observed to fill the entire body of the flagellate.

The Neuromotor System

Since the introduction of the conception of a neuromotor system (Kofoid and Christiansen, 1915*a*, *b*, Kofoid, 1916), subsequent investigations (Swezy, 1916; Boeck, 1917; Kofoid and Swezy, 1919*a*, *b*, 1920, 1922) have demonstrated the presence of such a system in many different flagellates. The neuromotor system consists essentially of the flagellum, the blepharoplast with its connected structures, and the rhizoplast extending from the nucleus to the blepharoplast, thus forming an integrated system composed of the nucleus and locomotor organelles. In addition there is, at the base of the nuclear rhizoplast, an extranuclear centrosome, the center of the system.

The system in *Menoidium* is simple: there is a single flagellum ending in a blepharoplast, from which a rhizoplast extends to the nucleus (fig. A; pl. 40, fig. 9). The blepharoplast is located at the bottom or side of a vacuole-like enlargement, the reservoir, at the posterior end of the gullet (fig. A). The nuclear rhizoplast is very delicate and can be seen in well-stained material only; Bordeaux red-iron haematoxylin preparations are most favorable for such observations. So far it has been impossible to trace the rhizoplast in flagellates in which the nucleus is at the posterior end. In many forms, other than euglenoids, there is an extranuclear centrosome at the base of the rhizoplast. In *Euglena agilis* (fig. B) a similarly located granule is easily detected in Bordeaux red-iron haematoxylin preparations; in *Menoidium* (fig. A) such a granule has been determined in similar preparations by the use of green-filtered monochromatic light; however, this granule in *Menoidium* has been found in comparatively few of the flagellates examined, since it is very small and does not always stain heavily. Thus it appears that the neuromotor system of at least some of the euglenoids is essentially similar to that of *Giardia* (Kofoid and Swezy, 1922), *Trichomonas* (Kofoid and Swezy, 1915*a*) and related forms, in that they possess an extranuclear centrosome at the base of the rhizoplast. Structures such as those described by Wager (1899) at the level of the eyespot in *Euglena* are not present in *Menoidium*.

Gullet and Reservoir

The gullet-reservoir structure in *Menoidium incurvum* (fig. A) is similar to that described for *Euglena* (Wager, 1899; Haase, 1910; Hamburger, 1911), *Astasia* (Bělař, 1916), *Peranema* (Hartmann and Chagas, 1910), *Heteronema* (Rhodes and Kirby, MSS), *Eutreptia* (Steuer, 1904), and *Copromonas* (Dobell, 1908). The gullet, opening somewhat eccentrically at the anterior end, is apparently con-

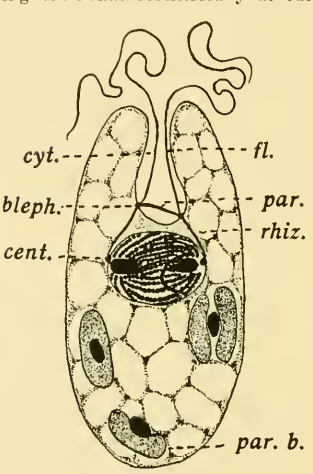

Fig. B

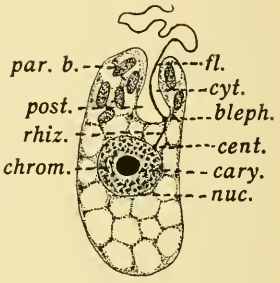

Fig. A

Fig. A. *Menoidium incurvum*, diagrammatic camera lucida sketch: *bleph.*, blepharoplast; *cary.*, endosome; *cent.*, extranuclear centrosome; *chrom.*, chromatin granules in resting nucleus; *cyt.*, gullet; *fl.*, flagellum; *nuc.*, nuclear membrane; *par. b.*, paramylum bodies; *post.*, reservoir; *rhiz.*, rhizoplast. × 2100.

Fig. B. *Euglena agilis*, camera lucida drawing: *bleph.*, blepharoplast; *cent.*, extranuclear centrosome; *cyt.*, gullet; *fl.*, flagellum; *par.*, paradesmose; *par. b.*, paramylum bodies; *rhiz.*, rhizoplast. × 2500.

tinuous with a reservoir situated usually near the nucleus (fig. A). A contractile vacuole in connection with the reservoir, as described in *Copromonas* (Dobell, 1908), *Euglena viridis* (Wager, 1899), and *Eutreptia* (Steuer, 1904), has not been observed in *Menoidium*. Since no ingestion of food particles has been observed and no typical food vacuoles, which are characteristic of holozoic forms, have been seen, the function of the cytostome and gullet is evidently not that of ingestion of solid food. Khawkine (1886) has suggested that the

gullet of *Euglena* is for the admission of liquid food. Wager (1899), however, considers this hypothesis as unproved, and states that the gullet and reservoir are excretory organelles, serving as outlet for substances discharged by the contractile vacuole. The regular occurrence of a number of paramylum bodies at the anterior end in *Menoidium* suggests that the gullet is in some way connected with metabolism, possibly through the admission of liquid food; for the liquid medium would probably enter the gullet unless there is some undiscovered means of keeping the cytostome closed. But whatever the function of the gullet and reservoir, the structure of these organelles is essentially similar in the holozoic, the saprozoic, and the holophytic euglenoids.

The Nucleus

The nucleus of *Menoidium* is usually situated near the center of the body, although frequently posterior and sometimes at the extreme posterior end. In structure the nucleus (fig. A) is quite typical for the euglenoids. There is a homogeneous, heavily-staining endosome (the "*Binnenkörper*" of Doflein and Tschenzoff) located usually near the center; the term endosome is used in the sense ascribed to it by Minchin (1912) and is not to be confused with the "entosome" of Prowazek (1901). Around the endosome, in the resting nucleus (pl. 40, fig. 7), chromatin granules occur apparently in the nodes of a linin network; in mitosis, the chromatin forms distinct chromosomes surrounding the endosome. In a few cases twelve have been counted, but this is only a tentative determination since the exact limits of the chromosomes are usually difficult to determine. A fairly distinct nuclear membrane is present, and persists throughout mitosis.

HABITS AND ACTIVITIES

The method of nutrition in *Menoidium* is probably saprozoic, since the evidence at hand favors such a conclusion; observation of the living organism has failed to reveal any ingestion of solid food particles, and no such particles have been seen in stained material. Lemmermann (1913) also regards *Menoidium incurvum* as "mesosaprob." *Menoidium* is preyed upon by the *Euplotes, Stylonychia*, and *Amoeba* which have usually been present in the cultures. The amoebae are especially voracious, often engulfing several of the flagellates, which persist for some time during digestion.

The locomotion of *Menoidium* is characteristic, since the smooth gliding type of movement found in many euglenoids is lacking. There is a rotation to the right, but this is accompanied by an oscillation of the body on its minor axis, producing an apparently awkward type of locomotion; yet this locomotion is evidently effective enough, since the flagellate is capable of traveling more rapidly than the much larger *Euglena* that occurs with it.

Although encysted forms are mentioned by Lemmermann (1913), the evidence for their occurrence in the cultures under observation is inconclusive. Several possible cases have been found in stained material, but owing to the fact that the cultures have contained several other flagellates positive identification of these cysts as those of *Menoidium* has not been possible. In this connection there might be mentioned a peculiar case of an individual which apparently contained three normal nuclei, one in the center of the body, the second anterior, and the third posterior, to the first; since this was the only case discovered, its significance has not been determined.

NUCLEAR DIVISION

The Resting Stage

The resting nucleus of *Menoidium incurvum* (pl. 40, fig. 7) contains a more or less centrally located endosome, around which the chromatin occurs in the nodes of a linin network. With the use of counterstains, such as Bordeaux red, after haematoxylin, the network is lightly stained, while the chromatin masses are deeply stained. This granular condition of the chromatin merges gradually into that of the coiled chromatin threads of the early prophase, so that it would be exceedingly difficult to determine just when the prophase begins.

Contrary to the observations of Bělař (1916), Hartmann and Chagas (1910), and Haase (1910) on various euglenoids, the endosome of *Menoidium* shows no centriole at this or any other stage of mitosis. My haematoxylin preparations vary from deeply stained to almost totally destained; also a number of anilin nuclear stains and counterstains after haematoxylin have been used; yet none of these brings out any structure resembling a centriole.

Prophase

With the initiation of the prophase, the scattered chromatin granules appear to become organized into fine thread-like structures, so that soon the semblance of a fine spireme is produced (pl. 40, fig. 8). It has not been determined with any degree of certainty how many threads this 'spireme' contains, but in figure 8 of plate 40 are shown what appear to be the ends of several threads, indicating the presence of more than one. The fine chromatin threads gradually shorten and thicken until, finally, distinct chromosomes are formed. Although it is possible that such structures exist, there is no evidence whatever for the emergence of chromosome pairs from the resting stage into the prophase, as described by Tschenzoff (1916) for *Euglena*. At first these chromosomes are coiled in a seemingly haphazard fashion, but they soon assume a somewhat radial arrangement with respect to the endosome (pl. 40, figs. 9 and 10). The number of these chromosomes of the prophase has not been definitely determined, since the exact endings of all of them cannot be made out; tentatively their number may be stated as approximately twelve. Except in a very few instances the chromosomes have not appeared granular, as described for *Euglena* (Tschenzoff, 1916; Dehorne, 1920) and *Heteronema* (Rhodes and Kirby, MSS). It is quite possible, however, that such is the structure of the chromosomes of *Menoidium;* their comparatively small size renders critical observation difficult.

With the advance of the prophase, the finer threads become transformed into the thicker chromosomes of the later stages (pl. 40, fig. 11). In the meantime, the endosome has begun to elongate in preparation for the metaphase, while the chromosomes still appear radially arranged with respect to the axis of the endosome.

Metaphase

With further elongation of the endosome, splitting of the chromosomes apparently begins (pl. 40, figs. 12 and 13; pl. 41, figs. 14 and 15). In such stages the chromosomes appear thinner and more numerous than those of the late prophase. Although the evidence is not entirely conclusive, the split apparently begins at one end of a thick chromosome and continues toward the other, resulting in the formation of a V-shaped structure (pl. 40, fig. 13; pl. 41, fig. 15). During the splitting process the free ends of the V's begin to migrate

toward opposite poles of the endosome. In the next stage observed, the chromosomes are arranged in the so-called equatorial plate (pl. 41, figs. 16, 17, 20, and 21), in which they are grouped around the endosome parallel to its long axis. From the observed behavior of the chromosomes, it seems probable that the equatorial plate is formed by an unfolding of the V-shaped chromosome pairs, the free ends of which have migrated to opposite poles of the endosome while the other ends remain attached in the plane of the equator.

Anaphase

In the early anaphase (pl. 41, fig. 18) the daughter chromosomes begin to separate in the equatorial plane, resulting at a slightly later stage (pl. 41, fig. 19) in complete separation of the two groups of daughter chromosomes. Further elongation of the nucleus now begins, with the chromosomes still retaining their parallel relation to the endosome. Constriction of the nucleus into two daughter nuclei occurs and these begin to pull apart, still connected by the middle portion of the endosome (pl. 41, figs. 22 and 23). This connecting portion becomes thinner and thinner as the nuclei become further separated, until finally constriction is completed at the end of the anaphase (pl. 41, fig. 24). The nuclear membrane remains intact throughout this stage.

Telophase

After the connecting thread of the endosome has broken (pl. 41, fig. 24), the daughter nuclei begin to round up (pl. 41, fig. 25). The two new endosomes shorten and reassume their spherical shape, and the parallel arrangement of the chromosomes is replaced by a spiral organization comparable with that of the prophase. This reorganization process is continued until typical resting nuclei are formed in the daughter flagellates with the completion of binary fission.

There is no adequate evidence here for a precocious splitting of the chromosomes in either the anaphase or the late telophase, as in *Euglena* (Tschenzoff, 1916). If such were the case, the metaphase phenomena would involve merely the separation of previously formed daughter chromosomes; but in *Menoidium incurvum* the actual splitting seems to occur in the metaphase.

BINARY FISSION

Binary fission is initiated by division of the blepharoplast, rhizoplast, and granule, or extranuclear centrosome, at the base of the rhizoplast; later two flagella are seen. Whether the second flagellum arises from the old by splitting, or as a new outgrowth from the second blepharoplast with the old flagellum retained by the first, could not be determined. Although outgrowth of the new flagellum is to be expected, there is some indication of splitting beginning at the blepharoplast and proceeding distally (pl. 40, figs. 10 and 12). It is more probable, however, that this appearance is produced by outgrowth of one flagellum in close conjunction with the other. A similar case is described by Kofoid and Christiansen (1915*b*) in *Giardia*, where the new flagella arise as outgrowths from the blepharoplast, each new flagellum following the intracytoplasmic path of the corresponding old one and emerging at its side. In *Menoidium* the two flagella are at first closely associated, as shown in figure 14 (pl. 41), in which the two blepharoplasts are still close together and the two flagella appear almost fused in most of their course through the gullet.

In *Heteronema acus*, Rhodes and Kirby (MSS) find that the new flagella arise as outgrowths. Dobell (1908) states that in *Copromonas* the old flagellum is drawn in on the approach of mitosis and, after the division of the blepharoplast, a new flagellum grows out from each daughter blepharoplast. Steuer (1904) records a splitting of the flagella in *Eutreptia*. Hartmann and Chagas (1910) describe the outgrowth of a new flagellum from each daughter blepharoplast in *Peranema*. Schüssler (1917) states that in *Scytomonas* the old flagellum may be retained by one daughter blepharoplast and the other produced by outgrowth, or that both flagella may arise by outgrowth as described by Dobell (1908). Thus, from the published accounts it would seem possible that the new flagella may be formed differently in the various euglenoids, but this is rather improbable; the origin of new flagella by outgrowth seems best supported.

Several observers (Wager, 1899; Hamburger, 1911) have described the flagella of various euglenoids as bifurcating, after entering the reservoir, into two fibers, each of which ends in a basal granule or blepharoplast. Haase (1910) figures the flagellum as bifurcating at the level of the eyespot, and the resulting fibers as passing back-

ward where they end posterior to the nucleus in a single basal granule. Since there are cases in *Menoidium* which present such an appearance as that described by Wager, and also instances in which the flagellum arises from only one blepharoplast, it seems quite possible that such a double condition is produced either by the splitting of an originally single structure, or else by the beginning of outgrowth of the new flagellum. Also, Dobell (1908), Berliner (1909), Bělař (1916), Schüssler (1917), and Rhodes and Kirby (MSS) find only one blepharoplast for each flagellum.

Paradesmose

As the blepharoplasts become further separated in the late metaphase and early anaphase, they are seen to be connected by a paradesmose (pl. 41, figs. 17–19). This structure was first described by Kofoid and Swezy (1915*a*) in *Trichomonas* as a "chromatic thread which lies *outside* of the nuclear membrane." Subsequent papers (Kofoid and Christiansen, 1915*a*, *b*; Kofoid and Swezy, 1915*b*, 1919*a*, *b*, *c*, 1920, 1922; Swezy, 1915*a*, *b*; Boeck, 1917; Cutler, 1919; Rhodes, 1919) have described such a structure in many different flagellates, and it is probable that a paradesmose will prove to be a common characteristic of mitosis in flagellates. It should be stated, however, that in most cases the paradesmose is an exceedingly delicate structure and is very difficult to stain and determine; hence, many observers have failed to find it.

In *Copromonas subtilis*, Dobell (1908, pl. 5, figs. 44 and 45) figures a fine strand connecting the two daughter 'basal granules' at division; this structure resembles very closely the paradesmose found in *Menoidium*, so that it is probably permissible to assume that *Copromonas* also possesses a paradesmose. Dobell, however, attributes no significance to this structure, merely stating that "the basal granule then divides, becoming dumb-bell shaped, and finally being constricted into two daughter-granules." Yet Dobell finds no rhizoplast in *Copromonas*. It is probable, however, that a more adequate staining technique would have disclosed such a structure, since Berliner (1909) figures a rhizoplast in *Copromonas major*.

In *Euglena agilis* (fig. B) I have recently found a paradesmose similar to that in *Menoidium;* here also this structure is spun out between the two blepharoplasts after their division.

In *Menoidium*, as stated above, the paradesmose is spun out between the two daughter blepharoplasts. In *Trichomonas* Kofoid and Swezy (1915*a*) describe in mitosis the division of each blepharoplast into two new structures, a basal granule (blepharoplast proper) and a centrosome; while the flagella and paradesmose remain attached to basal granules, or blepharoplasts proper, the centrosomes become located outside the nuclear membrane at opposite ends of the spindle and connected to the blepharoplasts by the nuclear rhizoplasts. In such a case as this the original blepharoplasts would be considered *centroblepharoplasts* (Kofoid and Swezy, 1919*a*), since they divide to form both centrosomes and blepharoplasts. In other flagellates, such as *Giardia* (Kofoid and Swezy, 1922) the blepharoplast is connected by a rhizoplast to a centrosome just outside the nuclear membrane; during mitosis in *Giardia* the paradesmose is drawn out between the two extranuclear centrosomes. Thus there are two types of paradesmoses, differing in location; in *Giardia*, the paradesmose connects the centrosomes, whereas in *Trichomonas*, in which the centrosome originates from the centroblepharoplast in mitosis, the paradesmose remains connected to the two blepharoplasts. It is quite evident that the paradesmoses of *Menoidium incurvum* and *Euglena agilis* are homologous with the type found in *Trichomonas*, in that both of them are drawn out between the two daughter blepharoplasts, rather than with the paradesmose seen in *Giardia*. The origin of the extranuclear centrosome from the blepharoplast (centroblepharoplast) at the beginning of mitosis has not yet been demonstrated in *Menoidium*, but from the essential similarity in structure to that of *Trichomonas* it might be expected that the blepharoplast of this flagellate may be shown eventually to be a centroblepharoplast.

Gullet and Reservoir

With the progress of mitosis, the gullet and reservoir begin to prepare for fission; the first step is the general widening of both structures (pl. 41, fig. 17). Then, as the flagella move farther apart the reservoir becomes divided into two lateral portions (pl. 41, figs. 16, 18, and 19), each of which is to give rise to a new reservoir. At a later stage (pl. 41, figs. 22–25), the median plane of fission has constricted the gullet and reservoir into two daughter structures, one in each new flagellate.

After the separation of the two daughter nuclei in the early telophase, binary fission is carried to completion and two daughter flagellates are formed. The flagellates appear to swim about actively throughout the whole process of mitosis. Since cysts could not be identified, there is no evidence for division during encystment, although it is quite possible that, as in *Euglena* (Tschenzoff, 1916; Zumstein, 1899), such a process may occur.

DISCUSSION

As Hartmann and Chagas (1910) have suggested, the euglenoid nucleus is probably more easily studied than that of any other group of flagellates; yet the literature contains very few good accounts such as that of Tschenzoff (1916), for example, and the different authors hold conflicting viewpoints with regard to the structure of the nucleus and the nature of nuclear division. Mitosis of one sort or another has been described by Klebs (1892), Blochmann (1894), Keuten (1895), Dangeard (1902), Prowazek (1903), Berliner (1909), Hartmann and Chagas (1910), Haase (1910), Bělař (1916), Tschenzoff (1916), Schüssler (1917), Dehorne (1920), and Gard (1920).

Amitosis has been described in a few forms, for example, *Eutreptia* (Steuer, 1904) and *Copromonas* [*Scytomonas?*] (Dobell, 1908). As for *Copromonas*, Berliner (1909) finds chromosome formation in well stained preparations of *Copromonas major;* also, Schüssler (1917) describes in *Scytomonas* chromosome formation in properly destained haematoxylin preparations, and states that an appearance of amitosis is seen in overstained preparations; however, these chromosomes were interpreted by Schüssler as being in the karyosome. Hence, it is quite possible that Dobell's account of amitosis in *Copromonas subtilis* is the result of inadequate staining technique. Dobell (Dobell and O'Connor, 1921) has recently modified his original designation of nuclear division in this form to "amitosis or a *simple form of mitosis.*"

Sexual phenomena have been described in a few instances (Weisse, 1856; Dobell, 1908; Berliner, 1909; Haase, 1910), but such descriptions do not rest upon *critical cytological evidence* and are not to be accepted without confirmation. In *Copromonas*, Dobell asserts that two flagellates become fused at their anterior ends, the union

extending gradually to complete fusion. Two "reduction divisions" occur, producing in each conjugant a "reduced" nucleus and two "reduction" nuclei, the latter comparable with the polar bodies of oogenesis; the "reduced" nuclei unite to form a "zygote nucleus." The flagellum of one flagellate is drawn in, while that of the other persists, serving to keep the precocious pair in locomotion. After fusion, this 'zygote' animal is said to have its choice of remaining a single large flagellate which will later divide, or of encysting. While it is probable that Dobell really observed an apparent fusion, the question remains as to whether or not such behavior is to be interpreted as isogamy. Occurring as it does under such conditions that "*Even in my most healthy cultures a number of monads always underwent degenerative changes and died*" (Dobell, 1908, p. 102), and that "*one can often observe degenerating monads side by side with perfectly healthy individuals in active division or conjugation*" (Dobell, 1908, p. 103), the interpretation of this process as conjugation must remain subject to criticism. Berliner's (1909) description of isogamy in *Copromonas major* is quite similar, so that to both of these reports Dobell's criticism (Dobell and O'Connor, 1921) of Woodcock's description of conjugation in *Cercomonas* might appropriately be applied:

> . . . believes he has observed conjugation in . . . I have not done so, and consider—from the account published—that there is little or no evidence that conjugation occurs. The phenomenon observed . . . appears rather to be an abortive or regressive fission. . . .

Weisse (1856) describes small individuals of *Euglena*, or "Spermatozoids," which penetrate the cysts of larger individuals of *Euglena*, thus effecting fertilization. Haase (1910) describes the formation of gametes in *Euglena sanguinea* by an intrakaryosomal mitosis, and the union of free gametes to form a zygote; the proof offered by her figures is entirely inadequate, however, and her interpretation is evidently at fault. With such little evidence for sexual behavior, the occurrence of sexual reproduction in the euglenoids must, for the present at least, remain doubtful. Other points of controversy have been the nature of the outer chromatin and its behavior in division, and the nature of the endosome—its function in division, and whether or not it contains a centriole.

The Resting Nucleus

Tschenzoff (1916) describes the resting nucleus of *Euglena viridis* as containing a "*Binnenkörper*" around which is found the

> chromatische Substanz in Form von feinsten Bröckchen und Fädchen. Manchmal tritt mehr bröckliche Beschaffenheit des Chromatins, manchmals feinfädige zutage. Manchmal die Chromatinkörnen anf einer Netwerk aufgereiht zu sein. . . . Ein geschlossenes Webenwerk konnte ich nicht feststellen, wenn auch das Vorhandsein eines solches zu vermuten ist.

His description agrees with that of Bělař (1916) for *Astasia*, Hartmann and Chagas (1910) for *Peranema*, Steuer (1904) for *Eutreptia*, and Bütschli (1883–1889) for euglenoids in general. Klebs (1883) seems to have mistaken a nucleus in the early prophase for the typical resting nucleus, since he describes a spireme structure in the nucleus of *Euglena*. Keuten (1895) has also described a prophase, since he mentions distinct chromosomes in the resting nucleus of *Euglena*. Dangeard (1902) considers the nucleus of *Euglena* and other euglenoids to contain a karyosome, around which is wound a long chromatin thread, the coils of which present the appearance of chromosomes. This concept of the euglenoid nucleus has recently been revived by Dehorne (1920) in his work on *Euglena*.

The structure of the nucleus in *Menoidium* is similar to that described for *Euglena* by Tschenzoff. In *Menoidium*, however, the nuclear membrane appears to persist throughout mitosis, while Tschenzoff states that in *Euglena*

> eine echte doppelkonturierte Membran ist indessen anscheinend nicht vorhanden, sie wird nur manchmal durch ein Kunstprodukt vorgetäuscht. Während des ganzen Prozesses der Kernteilung wird sie regelmassig vermisst.

Yet in his figures the outline of the nucleus stands out sharply from the cytoplasm, so that a membrane of some sort may well have been present. Dehorne (1920) finds a nuclear membrane persisting in rare cases only. Dobell (1908) describes the nucleus of *Copromonas* as bounded by a nuclear membrane and containing a karyosome, around which there is a clear zone containing "achromatic granules" and crossed by radial "linin threads"; his "achromatic granules" evidently correspond to the chromatin granules which give rise to the chromosomes in the mitosis of other euglenoids.

The Endosome

The endosome, or "*Binnenkörper*" (Doflein, Tschenzoff), is one of the least understood structures in the euglenoid nucleus. Some authors ascribe to it an inactive rôle in mitosis; others believe it to be active in initiating division; while a few have described it as containing a centriole. With a few exceptions, such as Berliner (1909), Haase (1910), Schüssler (1917), all are agreed that the endosome has nothing to do with the formation of chromosomes. Since Haase, however, describes also the formation of gametes by an intrakaryosomal mitosis and the union of gametes to form a zygote, her interpretation is open to suspicion.

The question of the presence or absence of a centriole in the endosome of euglenoids is not yet definitely settled. A centriole or centrosome has been described by Berliner (1909), Hartmann and Chagas (1910), Haase (1910), Bělař (1916), Schüssler (1917). Schüssler describes "*Centren*" in the middle of the "*Pseudopolkörper*," which are formed at the poles of the endosome; he reverts to the theory of chromatic dualism when he states that in

> allen genauer untersuchten einfacher Protozoenkernen sich stets zwei gesonderte Komponenten nachweisen lassen, eine lokomotorische und eine generative.

He considers that in the phylogeny of the euglenoids the more primitive types, represented by *Scytomonas*, have both nuclear components localized in the endosome, while in the higher forms such as *Euglena* the generative function is invested in the outer chromatin. Berliner (1909) believes that in the division of *Copromonas major* the old basal granule degenerates or is drawn back into the nucleus, and that new basal granules arise from the centrioles of the daughter nuclei; from these newly produced granules the flagella of the daughter flagellates grow out. This is denied by Schüssler, and Dobell (1908) also fails to find any indications of such a process. The cytological evidence of Haase (1910) for the presence of centrioles is quite as inadequate as that for her other fanciful interpretations, so that her account is of little value. Bělař (1916), however, has described and figured a structure resembling a centriole in the endosome of *Astasia levis*, and has traced it from the prophase through the anaphase. Hartmann and Chagas (1910) figure a divided centriole in the endosome of *Peranema* during the prophase, but they have not followed this structure through mitosis, as Bělař has done in *Astasia*, and their

cytological evidence seems insufficient; Alexeieff (1913) even accuses Hartmann and Chagas of arbitrarily selecting a pair of granules and calling them "centrioles," as other centriolists are said to have done.

The structure of the endosome in *Menoidium* supports the views of Tschenzoff and others, since there is no evidence at all of a centriole in the endosome. Since the only account worthy of careful consideration is that of Bělař (1916), the probabilities seem rather against the presence of a centriole in the endosome of *all* euglenoids.

Fragmentation of the endosome occurs in the mitosis of some euglenoids, such as *Euglena* (Haase, 1910; Dangeard, 1902) and *Heteronema* (Rhodes and Kirby, MSS) but such a phenomenon has not been discovered in *Menoidium*. The significance of such a process is not known.

Another interpretation of the endosome—the theory of the nucleolo-centrosome—was advanced by Keuten (1895). He compared the endosome ("*Nucleolo-centrosom*") with the centrosome and central spindle of the diatoms, and with the intranuclear centrosome of *Ascaris* (Brauer, 1893). This theory was supported by Schaudinn (1896), who cited an interesting experiment on *Oxyrrhis marina* as evidence of the nature of the endosome. In the nucleus of this form there is normally a large endosome; when the flagellates were placed in diluted sea-water the endosome left the nucleus and behaved as an extranuclear centrosome. Steuer (1904) accepts the term nucleolo-centrosome, but the more modern investigators have discarded this conception, employing such terms as karyosome, "*Binnenkörper*" (Tschenzoff, Doflein), and endosome (Minchin).

It has been suggested to me by Dr. Kofoid that a solution of the endosome problem is to be sought in connection with the intranuclear rhizoplast (Kofoid and Christiansen, 1915*b*; Boeck, 1917). In many flagellates activity within the nucleus is apparently correlated with the activity of the extranuclear centrosome; the physical bond between these two structures is the intranuclear rhizoplast, connecting the centrosome with the karyosome. There is a possibility that the endosome of the euglenoids may, in some way, be comparable with this intranuclear rhizoplast; future investigation may perhaps throw some light upon this phase of the question.

The Prophase

Tschenzoff (1916) describes the prophase as beginning in *Euglena viridis* when the chromatic substance begins to form distinct granules, which later become arranged in rows to form chromatic threads. He notes the appearance of an increasing number of chromatic granules which lie at the nuclear surface and appear to crop out into the cytoplasm, producing wrinkles in the nuclear membrane. These mysterious granules are believed either to break up and pass out into the cytoplasm, or else to take part in the formation of chromosomes. Later the chromosomes become arranged more or less radially around the endosome; with the elongation of this structure, the chromosomes come to form a cylindrical ring parallel to its long axis. Dehorne (1920), in a comparative study of the euglenoid and other protozoan nuclei, comes to the conclusion that, as Dangeard (1902) believed, the nucleus of *Euglena* contains in the prophase a continuous chromatin thread; this 'spireme' is said to be composed of granules. He bases his statement on the appearance of loops in the chromosomes and the fact that he is unable to find free ends; however, his figures do not afford sufficient evidence to convince one of the accuracy of such an interpretation.

In *Menoidium* these early processes are similar to those described by Tschenzoff, with the exception that no granules, such as he mentions, have been observed. In many cases structures resembling these granules of Tschenzoff have been seen, but they can always be resolved into optical cross-sections of chromatin threads. It is thus possible that Tschenzoff has mistaken optical sections of chromatin threads for actual granules; such an explanation might account for most of these structures shown in his figures.

In connection with the formation of chromosomes in the prophase, Sands (1922) presents some rather startling conclusions drawn from his work on *Tradescantia virginica*. He states that in the so-called resting stage, the genes attract to themselves "from the protoplasm, materials of a similar kind, thus molding next to the original gene another structure of similar parts, identically arranged, which then become bound together to form another gene, a replica of the first," and that these genes "after the genesis of their like, reassemble in such a fashion that they form a continuous spireme thread." He also states that the chromosomes are formed in the prophase by segmentation of a continuous spireme, and that no longitudinal splitting of the chromo-

somes occurs. His views are such a radical departure from the commonly accepted conception of the status of the chromosomes that they must be considered at present with some degree of skepticism, but they are, to say the least, very interesting and should provide an impetus to further critical investigation. His account, however, does not seem to be supported by the mitotic phenomena described for euglenoids.

The Metaphase

In *Euglena viridis*, according to Tschenzoff, the chromosomes, after being parallel to the endosome, come together in a thick equatorial zone, in which they are arranged at random with respect to the axis of the endosome. Separation of the double chromosomes observed at this stage begins at one end of the pairs, while the free ends begin to migrate toward opposite ends of the endosome. Dehorne (1920) finds in *Euglena*, at a stage approximating an early metaphase, distinct longitudinal cleavage of separate chromosomes—"*Le clivage est très surprenant chez un tel noyau, mais indubitable.*" At this stage he is unable to find a continuous spireme, but he believes that the free ends of the chromosomes later fuse again to form a single thread. This formation of separate chromosomes, their longitudinal cleavage, and the re-formation of the spireme occur prior to the anaphase, so that the separation of the equatorial belt into two daughter groups is due to a "division transversale" of the spireme. This interpretation of Dehorne is in marked contrast to that of Tschenzoff (1916), who finds in *Euglena viridis* longitudinal division of the chromosomes in a true mitosis, and no indications of a continuous spireme. When one considers that Dehorne, in the paper under consideration, is bent upon tracing similarities in the nuclei of ciliates, euglenoids, and Cyanophyceae, his interpretations may be suspected to have been influenced somewhat by his underlying hypothesis.

From the behavior of the chromosomes in *Menoidium*, it is concluded that the procedure in the metaphase is slightly different from that described by Tschenzoff for *Euglena*. The thickened chromosomes of the late prophase appear to remain radially arranged with respect to the axis of the elongating endosome. In the early metaphase, splitting apparently begins at one pole of these thick chromosomes and proceeds toward the other, while the free ends of the daughter chromosomes begin to move toward opposite ends of the endosome. Although no evidence for such a condition has been

found, these chromosomes of the late prophase might, in reality, be paired chromosomes which have already split at an earlier stage, as described by Tschenzoff in *Euglena viridis;* if such were the case, the metaphase "splitting' would be merely the separation of previously formed daughter chromosomes. Tschenzoff finds that in *Euglena* the chromosomes split in the telophase or late anaphase, while the daughter chromosomes so formed pair in the prophase of the next mitosis and are permanently separated in the metaphase. Such a process could not be confirmed in *Menoidium;* however, it should be stated that the nucleus of this flagellate is much smaller than that of *Euglena*, so that it may be that such a process occurs and has not yet been detected.

The migration of the free ends of the V-shaped chromosome pairs during the metaphase in *Menoidium* results in the formation of the ring of chromosomes parallel to the endosome ("equatorial plate stage"); in such a case the ring is formed by the paired daughter chromosomes which have unfolded but still remain attached at the equator. This is supported by the condition shown in plate 41, figures 16 and 17, in which a split has not yet appeared in the plane of the equator through the end-to-end union of the daughter chromosomes. In slightly later stages (pl. 41, figs. 18 and 19) a split appears, while the chromosomes are still parallel to the endosome. A similar process of equatorial plate formation is described by Kofoid and Swezy (1919*b*) in *Trichonympha campanula:*

> . . . with the beginning of the formation of the spindle fibres, or somewhat earlier, another change takes place in the chromosomes, the loops straightening out so that the chromosomes come to lie parallel to the paradesmose. . . . The completion of this gives the equatorial plate phase, with the chromosomes still joined by an end to end union in the equatorial plane.

Anaphase and Telophase

Tschenzoff's description of the later stages of mitosis in *Euglena* is in accord with the observed phenomena in *Menoidium*, except for the fact that a splitting of the chromosomes in the telophase or late anaphase could not be determined in the latter. Dehorne (1920) states that, in the late anaphase, the separate segments of the spireme produced by the transverse division of the metaphase stage reunite to form the continuous spireme characteristic of the prophase; in such flagellates as *Euglena*, however, where the chromosomes are closely massed together in the anaphase, it is difficult to understand how one could be certain that such a structure exists.

SUMMARY

Menoidium incurvum is a small saprozoic euglenoid characterized by a rigid body marked on the surface with from ten to fifteen widely separated longitudinal striations; the body contains a number of nearly colorless plastids, or paramylum bodies, located usually at the anterior end, although occasionally at both ends. The gullet and reservoir are similar in structure to those of other euglenoids, but are not used for ingestion of solid food.

The neuromotor apparatus is a simple system consisting of a flagellum ending in a blepharoplast, from which a rhizoplast extends to the nucleus. The granule, or centrosome, at the base of the rhizoplast in *Menoidium*, and also in *Euglena agilis*, is similar to the extranuclear centrosome of such forms as *Giardia* and *Trichomonas*. During mitosis the daughter blepharoplasts remain connected for a time by a paradesmose; this is similar in both *Menoidium* and *Euglena agilis* to that of *Trichomonas*. There is some indication of a splitting of the old flagellum instead of the expected outgrowth in mitosis, but it is more probable that there is an outgrowth of the new flagellum in close conjunction with the old.

The resting nucleus contains an endosome surrounded by chromatin granules in the nodes of a linin network; during mitosis these granules become organized into distinct chromosomes, whose number is tentatively determined to be twelve. The equatorial ring or plate of the late metaphase is apparently produced by an unfolding of the V-shaped chromosome pairs formed by the metaphase split; the free ends of the V's migrate to opposite poles of the endosome, leaving their other ends attached in the plane of the equator. At this stage the chromosomes are parallel to the long axis of the endosome, and they remain so until the late anaphase. Splitting of the chromosomes occurs in the metaphase; a precocious splitting in the preceding telophase is possible, but there is no evidence whatever that this occurs. The endosome contains no centriole at any stage of mitosis. The nuclear membrane persists throughout nuclear division.

Encysted forms of *Menoidium* have not been identified in either living or stained material.

LITERATURE CITED

Alexeieff, A.

1911. Haplomitose chez les Euglénieus et dans d'autres groupes de protozoaires. C. R. Soc. Biol., Paris, **71**, 614, 8 figs. in text.

1913. Systematisation de la mitose dite "primitive." Sur la question du centriole. Arch. f. Prot., **29**, 344–363, 7 figs. in text.

Belar, K.

1916. Protozoenstudien. I.—*Amoeba diplogena, Astasia levis* n. sp., *Rhynchomonas nasuta* Klebs. Arch. f. Prot., **36**, 13–51, pls. 2–4, 3 figs. in text.

Berliner, E.

1909. Flagellatenstudien. Arch. f. Prot., **15**, 297–326, pls. 28–29.

Blochmann, R.

1894. Kleine Mitteilungen über Protozoen. Über die Kernteilung bei *Euglena*. Biol. Centralbl., **14**, 81–91, 3 figs. in text.

Boeck, W. C.

1917. Mitosis in *Giardia microti*. Univ. Calif. Publ. Zool., **18**, 1–26, pl. 1.

Brauer, A.

1893. Zur Kenntnis der Spermatogenese von *Ascaris megalocephala*. Arch. f. mikr. Anat., **42**, 153–213, 3 pls.

Bütschli, O.

1878. Beitrage zur Kenntnis der Flagellaten und einiger verwandter Organismen. Ztschr. f. wiss. Zool., **30**, 205–281, pls. 11–15.

1883–89. "Protozoa," *in* Bronn, Klassen und Ordnung des Thierreichs., **1**, 1–2035, pls. 1–79.

Chatton, E.

1910. Essai sur la structure du noyau et la mitose chez les Amoebiens. Faits et théories. Arch. de Zool. expér. et gén., 5e ser., **5**, 267–337, 13 figs. in text.

Cutler, D. W.

1919. Observations on the Protozoa parasitic in the hindgut of *Archotermopsis wroughtoni* Desn.: Part I.—*Ditrichomonas termitis*, n.g., n.sp., Quar. Jour. Micr. Sci., **63**, 555–588, pls. 31–33.

Dangeard, P. A.

1902. Recherches sur les Euglénieus. Le Botaniste, 8e ser., **1902**, 96–357, pls. 1–4.

Dehorne, A.

1920. Contribution à l'étude comparée de l'appareil nucléaire des Infusiores ciliés (*Paramecium caudatum* et *Colpidium truncatum*), des Euglénieus et des Cyanophycées. Arch. Zool. expér. et gén., **60**, 47–176, pls. 1–4.

Dobell, C. C.

1908. The structure and life-history of *Copromonas subtilis* n.g., n.sp.: A contribution to our knowledge of the Flagellata. Quar. Jour. Micr. Sci., **52**, 75–120, pls. 4–5, 3 figs. in text.

Dobell, C., and O'Connor, F. W.
1921. The intestinal Protozoa of man (London, J. Bales, Son and Danielsson), ix + 211 pp., 8 pls.

Doflein, F.
1916. Lehrbuch der Protozoenkunde (Jena, G. Fischer), xiv + 1190 pp., 1198 figs. in text.

Fresenius, G.
1858. Beiträge zur Kenntnis mikroskopischer Organismen. Abh. Senck. Naturf. Gesell., **2**, 211–242, pls. 10–12.

Gard, M.
1920. Division chez *Euglena limosa*. C. R. Acad Sci., Paris, **170**, 291–292.

Gicklhorn, J.
1921. Eine einfache Methode zur Herstellung der Geissel mit Basalkorn bei Flagellaten, besonders bei Euglenien. Ztschr. f. wiss. Mikr., **38**, 123–129.

Haase, G.
1910. Studien über *Euglena sanguinea*. Arch. f. Prot., **20**, 47–59, pls. 4–6.

Hamburger, C.
1911. Studien über *Euglena Ehrenbergii*, insbesondere über die Körperhülle. Sitz.-Ber. d. Heidelburg. Akad. wiss. Math.-naturw. Kl., Jahrg. **1911**, 4 Abh., 1–22 pp., 1 pl.

Hartmann, M., and Chagas, C.
1910. Flagellatenstudien. Mem. Inst. Osw. Cruz, **2**, 64–125, pls. 4–9.

Keuten, J.
1895. Die Kernteilung von *Euglena viridis* Ehrenb. Ztschr. f. wiss. Zool., **60**, 215–235, pl. 11.

Khawkine, W.
1886. Recherches biologiques sur l'*Astasia ocellata* n.s. et l'*Euglena viridis* Ehr. Seconde Partie. *Euglena viridis*. Ann. Sci. Nat., Zool., 7e Ser., **1**, No. 6, pp. 319–376, pl. 16.

Klebs, G.
1883. Über die Organisation einiger Flagellatengruppen und ihre Beziehungen zu Algen und Infusorien. Unters. bot. Inst. Tübingen, **1**, 233–325.
1892. Flagellatenstudien. Theil II. Ztschr. f. wiss Zool., **55**, 353–445, pls. 17–18.

Kofoid, C. A.
1916. The biological and medical significance of the intestinal flagellates. Proc. 2d Pan-American Sci. Cong., **10**, 546–565.

Kofoid, C. A., and Christiansen, E. B.
1915*a*. On the life-history of *Giardia*. Proc. Nat. Acad. Sci., **1**, 547–552, 1 fig. in text.
1915*b*. On binary and multiple fission in *Giardia muris* (Grassi). Univ. Calif. Publ. Zool., **16**, 30–54, pls. 5–8, 1 fig. in text.

Kofoid, C. A., and Swezy, O.
1915*a*. Mitosis in *Trichomonas*. Proc. Nat. Acad. Sci., **1**, 315–321, 9 figs. in text.
1915*b*. Mitosis and multiple fission in trichomonad flagellates. Proc. Am. Acad. Arts and Sci., Boston, **2**, 289–378, pls. 1–8, 7 figs. in text.

1919*a*. Studies on the parasites of termites. II. On *Trichomitus termitidis*, a polymastigote flagellate with a highly developed neuromotor system. Univ. Calif. Publ. Zool., **20**, 21–40, pls. 3–4, 2 figs. in text.

1919*b*. Studies on the parasites of termites. III. On *Trichonympha campanula*, sp. nov. Univ. Calif. Publ. Zool., **20**, 41–98, pls. 2–12, 4 figs. in text.

1919c. Studies on the parasites of termites. IV. On *Leidyopsis sphaerica* gen. nov., sp. nov. Univ. Calif. Publ. Zool., **20**, 99–116, pls. 13–14, 1 fig. in text.

1920. On the morphology and mitosis of *Chilomastix mesnili* (Wenyon), a common flagellate of the human intestine. Univ. Calif. Publ. Zool., **20**, 117–144, pls. 15–17, 2 figs. in text.

1922. Mitosis and fission in the active and encysted phases of *Giardia enterica* (Grassi) of man, with a discussion of the origin of bilateral symmetry in the polymastigote flagellates. Univ. Calif. Publ. Zool., **20**, 199–234, pls. 23–26, 11 figs. in text.

Lemmermann, E.

1913. "Eugleninae" *in* Die Süsswasserflora Deutschlands, Österreichs und der Schweiz, Heft 2 (Jena, G. Fischer), pp. 115–174, figs. 181–377.

Prowazek, S. von.

1901. Kernteilung und Vermehrung der *Polytoma*. Öst. bot. Ztschr., **51**, 51–70. (Original article not consulted.)

1903. Die Kernteilung von *Entosiphon*. Arch. f. Prot., **2**, 325–328, 12 figs. in text.

Rhodes, R. C.

1919. Binary fission in *Collodictyon triciliatum* Carter. Univ. Calif. Publ. Zool., **19**, 201–274, pls. 7–14, 4 figs. in text.

Rhodes, R. C., and Kirby, H.

Mitosis in *Heteronema acus*. MSS.

Sands, H. C.

1922. Perigenesis. Science, **56**, 517–518.

Schaudinn, F.

1896. Über das Centralkorn der Heliozoen, ein Beitrag zur Centrosomenfrage. Verh. deutsch. Zool. Gesell. (Bonn), **1896**, 113–130.

Schüssler, H.

1917. Cytologische und entwicklungsgeschichtliche Protozoenstudien. I. Über die Teilung von *Scytomonas pusilla* Stein. Arch f. Prot., **38**, 117–125, pl. 5.

Seligo, A.

1885. Untersuchungen über Flagellaten. Cohn's Beitr. zur Biol., **4**, 145–182, pl. 8.

Stein, F. R. v.

1878. Der Organismus der Infusionsthiere. Abth. III. Der Organismus der Flagellaten, Hälfte I (Leipzig, Engelmann), x + 154 pp., pls. 1–24.

Steuer, A.

1904. Über eine Euglenoide (*Eutreptia*) aus dem Canale Grande von Triest. Arch. f. Prot., **3**, 126–137, 13 figs. in text.

Swezy, O.

1915*a*. Binary and multiple fission in *Hexamitus*. Univ. Calif. Publ. Zool., **16**, 71–88, pls. 9–11.

1915*b*. On a new trichomonas flagellate, *Trichomitus parvus*, from the intestine of Amphibians. Univ. Calif. Publ. Zool., **16**, 89–94, pl. 12.

1916. On the genera *Monocercomonas* and *Polymastix*. Univ. Calif. Publ. Zool., **16**, 127–138, pls. 16–17.

Tschenzoff, B.

1916. Die Kernteilung bei *Euglena viridis* Ehrbg. Arch. f. Prot., **36**, 137–173, pls. 11–12, 2 figs. in text.

Wager, H.

1899. On the eyespot and flagellum in *Euglena viridis*. Jour. Linn. Soc. London, Botany, **27**, 463–481, pl. 32.

Weisse,

1856. Über den Lebenslauf der Euglenen. Bull. Phys. Math. de l'Acad. Imp. d. St. Petersburg, **12**. (Original article not available.)

Zumstein, H.

1899. Zur Morphologie und Physiologie der *Euglena gracilis*. Jahrb. f. wiss. Bot., **34**, 149–196, pl. 6.

EXPLANATION OF PLATES

Figures 6–12, 14, 16–19, 22, and 25 are drawn from material killed in Schaudinn's fluid and stained in 0.1 per cent Bordeaux red preceding iron-alum haematoxylin. Magnification × 2100 (except fig. 2).

PLATE 40

Fig. 1. Camera lucida sketch of unstained flagellate, showing longitudinal striations, plastids, gullet, and flagellum.

Fig. 2. Diagrammatic enlargement of a camera lucida sketch. Note increase in number of striations with the preparation for binary fission.

Fig. 3. Posterior end of flagellate showing fourteen striations converging to a point.

Fig. 4. Anterior end; striations extend to edge of cytostome.

Fig. 5. Optical section through posterior end; the striations appear to be elevated ridges in the pellicle.

Fig. 6. Unstained flagellate, showing plastids at both anterior and posterior ends.

Fig. 7. Resting nucleus, showing aggregation of chromatin particles as the prophase approaches.

Fig. 8. Very early prophase; chromatin in form of fine threads irregularly coiled around the endosome. The ends of several threads can be seen.

Fig. 9. Nucleus in prophase; chromosomes show radial grouping around endosome. Flagellum, blepharoplast, and rhizoplast are also shown.

Fig. 10. Surface view of nucleus at about same stage as that shown in figure 9. Note appearance of two blepharoplasts; only one rhizoplast could be traced.

Fig. 11. Nucleus shows thicker chromosomes of later prophase.

Fig. 12. Note the two blepharoplasts and rhizoplasts and the apparent splitting of the proximal part of the flagellum; this probably represents the beginning of outgrowth of the new flagellum. Chromosomes are still radially arranged, but appear to have increased in number with the beginning of metaphase splitting.

Fig. 13. Later stage than figure 12. Nucleus shown in end view at about same stage shown in figures 14 and 15. Several V-shaped chromosome structures are seen.

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PLATE 41

Fig. 14. Semi-composite view of nucleus in metaphase; the endosome has elongated, and its upper end is at a deeper focal plane than the lower. The rhizoplasts could not be traced from the blepharoplasts.

Fig. 15. Nucleus elongated in later metaphase; endosome now extends from one end of the nucleus to the other, and the free ends of the chromosome pairs have begun to migrate.

Fig. 16. Nucleus in late metaphase, or 'equatorial plate' stage. The free ends of the daughter chromosomes have now reached the poles of the endosome, but the other ends are still attached at the equator. The reservoir and gullet have widened with the migration of blepharoplasts and flagella.

Fig. 17. Nucleus in 'equatorial plate' stage. The blepharoplasts are connected by a paradesmose, and from each a rhizoplast extends to the nucleus.

Fig. 18. The ends of the daughter chromosomes are beginning to separate at the equator in the early anaphase. Blepharoplasts still connected by the paradesmose.

Fig. 19. Nucleus in early anaphase, showing complete separation of the daughter chromosomes; the daughter groups are connected by the middle portion of the endosome. Blepharoplasts still connected by the paradesmose.

Fig. 20. End view of nucleus at approximately the stage shown in figures 17 and 18.

Fig. 21. Optical section of the nucleus shown in figure 20, at a plane about one-third the distance from the upper end. Note, with figure 20, the parallel arrangement of the chromosomes.

Fig. 22. Later anaphase; the daughter nuclei are distinctly constricted; the chromosomes are still parallel to the endosome.

Fig. 23. Later anaphase, showing further separation of the daughter nuclei.

Fig. 24. Early telophase. Constriction is just completed. The chromosomes of the daughter nuclei are still parallel to the endosomes.

Fig. 25. Later telophase. The daughter nuclei have rounded up and regeneration has begun. Cell division is nearly completed.

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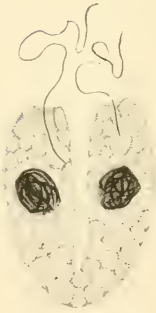
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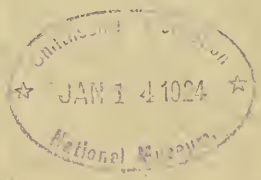
BY

EDNA HANNIBAL WAGENER



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Index, pp. 471–480.

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EDNA HANNIBAL WAGENER

INTRODUCTION

The cutaneous reaction has been so largely and successfully used in demonstrating hypersensitiveness to protein derivatives of both fungi and bacteria that it seemed possible that a similar reaction might be obtained with protein derivatives of protozoans. Some work has been done in this field of investigation, but thus far no conclusive results have been published. (See discussion at close of this paper.)

The selection of a favorable protozoan for experimentation offered several difficulties as apparently none of the intestinal forms has been grown in pure culture. Finally, however, *Leishmania* was found to fulfil the requirement; also to grow readily in quantity sufficient for the essential experimental tests with derived proteins.

ACKNOWLEDGMENTS

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MATERIAL AND TECHNIQUE

Cultures of *Leishmania tropica* and *Leishmania infantum* were grown on N.N.N. media made up in the proportions of 1.4 per cent agar and 0.5 per cent sodium chloride in distilled water. This was adjusted to pH 7.8, was tubed, autoclaved, and stored until needed. One day before being used 30 per cent of defibrinated rabbit blood was added and the tubes incubated overnight, to test them for sterility of the culture media. The tubes were then inoculated and the cotton plugs covered with rubber caps to prevent evaporation. After 10 to 12 days' incubation at room temperature, the surface of the media was found to be covered with minute, dewdrop colonies of the flagellates. This growth was rubbed off with a platinum loop into 1 c.c. of normal saline and decanted into a centrifuge tube. The washings from 15 to 20 cultures of each species were centrifuged separately for half an hour at 1800 revolutions a minute and the supernatant fluid decanted off. The organisms in the bottoms of the tubes were then diluted with normal saline to which 0.25 per cent phenol had been added until each cubic centimeter contained 1,500,000 organisms by direct count in a blood-counting chamber.

With the suspension thus prepared, two rabbits were immunized to each of the two species of *Leishmania* by intravenous injections of 2 c.c. each at three day intervals. Seven days after the last injection the rabbits were bled from the ear and macroscopic tests made for the presence of agglutinins. All four rabbits showed complete agglutination of the specific flagellate at a 1 to 980 dilution of the serum. These results were considerably higher than those of Bandi (1913), who found that immunized rabbits would agglutinate the specific strains of *Leishmania infantum* at a 1 to 160 dilution of the serum.

A leishmaniosin was prepared, following the technique used by Force and Stevens (1917) in the preparation of typhoidin. This consisted in washing off the growth from about 50 cultures of each species with 1 c.c. of saline, precipitating the protein with 95 per cent alcohol, washing with ether, and drying the residue over concentrated sulphuric acid in vacuum. As a small amount of haemoglobin from the culture media was dissolved in the saline, a control was prepared from washings from sterile culture media. About 0.1 gm. of dry

powder was obtained from each species and from the control. Unlike the typhoidin, however, these dry powders would not go into solution in saline, and no reaction occurred in the rabbits from the intradermal injection of 0.2 c.c. of the suspension of 1 part of powder in 100 parts of saline. It was therefore decided to grind each of the powders in a mortar, while adding slowly a weakly alkaline solution such as that recommended by Coca (1922) for the extraction of proteins from pollens and danders. So 100 parts of the extracting fluid of Coca, consisting of sodium chloride 0.5 per cent, sodium bicarbonate 0.05 per cent, and carbolic acid 0.4 per cent, in sterile distilled water were added to one part of dry powder. The suspensions were then placed in sterile containers, covered with toluol, and allowed to stand at room temperature for three days. Unfortunately, there was not a sufficient quantity of the extracted proteins to enable us to follow Coca's technique farther and determine the nitrogen content of the extract before using it. These alcoholic extracts will be designated alcoholic extracts of *L. tropica* and *L. infantum*.

At the same time a number of cultures of each species of *Leishmania* were washed off in saline, the washings centrifuged, and the protozoans in the bottom of the tube diluted with Coca's extracting fluid until 1 c.c. contained 2,000,000 organisms. These suspensions, spoken of hereafter as alkaline extracts of *L. tropica* and *L. infantum*, were also covered with toluol and allowed to stand at room temperature for three days. The alcoholic and alkaline extracts were then centrifuged at high speed for half an hour, and the supernatant fluid decanted into sterile tubes.

One normal and four immune rabbits which had been shaved on the back were inoculated intradermally with 0.2 c.c. of each extract and with a control of the extracting fluid to which a little toluol had been added. All extracts were tested for their sterility before being used for the inoculations.

The extracts prepared from the protein precipitated by means of alcohol gave unsatisfactory results. A reddened and occasionally a slightly indurated papule, indistinguishable from the control of that series, appeared within 24 hours and then disappeared after 48 hours. The alkaline extracts of *L. tropica* and *L. infantum*, on the other hand, produced at the end of 24 hours a small reddened papule which reached its height in 48 hours and persisted for five days. The center of this papule in all the immune animals consisted of an irregular ivory-white area 1 to 2 mm. in diameter, surrounded by an

TABLE 1

REACTION OF CUTANEOUS TESTS TO THE ALCOHOLIC AND ALKALINE EXTRACTS OF L. TROPICA AND L. INFANTUM

Rabbit	Immunized to	Extract	Dose in c. c.	L. tropica			L. infantum			Control			Result
				Redness in mm.	Red and induration	White necrotic	Redness	Red and induration	White necrotic	Redness	Red and induration	White necrotic	
22	*L. tropica*	Alcoholic	0.2	3	....	..	3	....	....	1	....	.	Negative
	L. tropica	Alkaline	0.2	5	5	1	3	3	1	(3)	....	.	Positive
10	*L. tropica*	Alcoholic	0.2	6	6	....	8	7	....	6	6	..	Negative
	L. tropica	Alkaline	0.2	5	5	2	5	5	3	(2)	....	. .	Positive
7	*L. infantum*	Alcoholic	0.2	10	10	..	7	7	. .	5	5	.	Negative
	L. infantum	Alkaline	0.2	*14*	*7*	2	*10*	*9*	2	(8)	....	..	Positive
127	*L. infantum*	Alcoholic	0.2	2	..	..	2	....	..	2	....	. .	Negative
	L. infantum	Alkaline	0.2	8	6	2	6	6	2	(3)	....	....	Positive
20	Normal	Alcoholic	0.2	3	. .	....	2	....	....	2	....	....	Negative
	Normal	Alkaline	0.2	2	. .	....	3	..	..	2	....	....	Negative

Numbers in parentheses indicate slight reaction.
Numbers italicized indicate marked reaction.

irregularly shaped red halo, which faded to a flesh color beyond the edge of the indurated area (pl. 42, fig. 4). The areas of induration varied from 5 to 7 mm. in diameter on the different immune rabbits. At about the fifth day these white centers formed a hard, yellowish brown crust, which sloughed off three or four days later without leaving a scar.

Neither the white center nor the area of induration was present in the control tests made on each rabbit with the extracting fluid (pl. 42, fig. 4*b*) or in any of the tests on the normal rabbit.

This reaction is comparable to that produced in tubercular animals by the intradermal injection of Old Tuberculin, which results in a red and indurated papule with a central necrotic area which sloughs off several days after the injection.

Krause and Peters (1920) demonstrated a local reaction to tuberculosis re-infection when living tubercle bacilli were injected intracutaneously into guinea pigs. This reaction was characterized by a reddened papule, rapid ulceration, and slough followed by healing. These authors considered this an immune reaction in the guinea pigs. As this reaction corresponds very closely to that obtained in the rabbits immunized to *Leishmania*, an attempt was made to further correlate the results by injecting into an immune and a normal rabbit cultures of the *living* flagellates. These injections were made intradermally on the testicle, this site being chosen on account of the thinness of the epidermis and also because Laveran (1917) had found it possible to produce Bagdad boils in white mice by injecting cultures of *Leishmania tropica* into the testicular sac. The rabbits were observed daily for a week and frequently thereafter. No reaction occurred. The reason, probably, was because, as suggested by Delanoe (1911), the living flagellates were being rapidly phagocytized.

One week after the first series of skin tests had been applied to the immune rabbits, they were retested in order to standardize more definitely the alkaline extracts of *L. tropica* and *L. infantum*. This was considered necessary because throughout the first tests the extract from *L. infantum* had produced in general a less marked reaction than that from *L. tropica*. This difference might have been due to an unequal concentration of protein because of the slightly larger size of *L. tropica* as compared with *L. infantum*. Hence extracts made by the addition of a definite number of flagellates per c.c. would not necessarily represent equivalent amounts of protein. Flagellate

TABLE 2

TESTS ON EFFECT OF DILUTION OF STOCK ALKALIN EXTRACTS OF L. TROPICA AND L. INFANTUM

Rabbit	Immunized to	Dilution of stock alkalin extracts	Dose in c. c.	L. tropica			L. infantum			Control			Results
				Redness mm.	Red and induration mm.	White necrotic mm.	Redness in mm.	Red and induration mm.	White necrotic mm.	Redness in mm.	Red and induration mm.	White necrotic	
22	*L. tropica*	Undil.	0.2	9	9	2	7	7	1	2	....	....	Positive
22	*L. tropica*	50%	0.2	8	8	2	4	4	1				Positive
10	*L. tropica*	Undil.	0.2	8	7	1	7	6	1	3	....	....	Positive
10	*L. tropica*	50%	0.2	4	4	1	5	4	1				Positive
7	*L. infantum*	Undil.	0.2	19	10	3	11	9	2	2	....	. .	Positive
7	*L. infantum*	50%	0.2	7	7	1	7	5	1				Positive
21	Normal	Undil.	0.2	2	....	....	1	..	..	2	....		Negative
21	Normal	50%	0.2	1	....	....	1	. .	. ..				

growths from a number of cultures were therefore washed off in saline and packed by rapid centrifugalization for one half-hour. One part of the packed organisms was then diluted with four parts of the alkaline extracting fluid, treated as before, and stored in the ice box. It was then possible to make up any dilution desired from these stock alkaline extracts of *L. tropica* and *L. infantum*.

Tests made by the intradermal injection of 0.2 c.c. of these stock alkaline extracts into the immune rabbits showed both of them to be unduly toxic (pl. 42, figs. 3*b* and *d*). When diluted 50 per cent with extracting fluid, however, the toxicity of both was greatly reduced, though a red and indurated papule more than 5 mm. in diameter, easily distinguishable from the control test and from the tests on the normal rabbit, was still produced (pl. 42, figs. 1 and 2). The difference in the size of the papule produced by *L. tropica* and by *L. infantum* persisted. The cause of this difference is, as yet, obscure.

The results of these tests are as shown in table 2.

DISCUSSION

It is probable that the immunization of rabbits to *Leishmania* produces in them the condition classified by Coca (1923) as *hypersensitiveness of infection*. Hypersensitiveness to derivatives of bacteria has been demonstrated by a number of investigators through the use of the intradermal test. Von Pirquet (1911) showed that the intradermal injection of vaccine lymph produces, in previously vaccinated persons, marked papules after 48 hours. Force and Stevens (1917) have described in detail the papule produced by the intradermal injection of typhoidin in typhoid immune rabbits. The intradermal tuberculin reaction in both man and animals is so generally known that a resumé of the literature is scarcely necessary. Recently Fairley (1923) found that the Casoni intradermal reaction gave positive results in 57.9 per cent of patients with proved hydatid infestation. This intradermal reaction is not constant in all cases giving positive complement fixation or precipitin reactions; in cases of ruptured cysts, this is due to the desensitization of the patient. Lanfranchi and Sani (1921) produced, with antigens prepared from *Trypanosoma brucei*, an ophthalmic reaction which persisted 36 hours

in horses infected with dourine. Normal horses showed a reaction persisting from 20 to 24 hours. The authors considered the reactions in the infected animals to be similar to those obtained in positive mallein reactions. Nusshag (1921) described inconclusive results obtained in dourine by the intradermal injection of .25 cc. of antigen prepared from trypanosomes. A faintly reddish papule appeared in 5 hours, reached its height in 18 hours, and disappeared within 24 hours. In the light of the tuberculin and typhoidin reactions, these results could scarcely be considered positive, but rather as a non-specific protein reaction. Force and Stevens (1917) consider specific only those intradermal reactions which reach their height at 48 hours and persist for at least 72 hours. The work of De Gaspari (1921) on dourine has been inaccessible. The reactions obtained by the intradermal injection of the alkaline extracts of *L. tropica* and *L. infantum* in immune rabbits are without doubt comparable to those obtained with tuberculin, typhoidin, and vaccine lymph.

It is of importance to note that rabbits immunized to one species of *Leishmania* are sensitive to the protein of both species, *L. tropica* producing the most pronounced reaction even in animals immunized to *L. infantum*. Hamman and Wolman (1912) have found that both human and bovine Old Tuberculin produced approximately the same sized papule in 127 out of 150 cases of tuberculosis tested. Meyer and Christiansen (1917) stated that some typhoid immune rabbits show non-specific reactions with paratyphoid and *B. coli* extracts which can be considered as group reactions.

These group reactions do not impair the diagnostic value of the skin reaction as a means of detecting hypersensitiveness to protozoan proteins. At the present time insufficient work has been done to determine the value of these tests in man or in animals infected with protozoa. It is hoped, however, that the results of this investigation may be made the basis for similar tests on cases or convalescents from leishmaniosis.

SUMMARY

The intradermal injection of alkaline extracts of *Leishmania tropica* or *Leishmania infantum* will produce a local reaction in the skin of a sensitized rabbit. This reaction is characterized by an erythematous papule which reaches its height in 48 hours and persists from 72 hours to 5 days.

The injection of a concentrated extract produces necrosis and sloughing. Normal rabbits show no reaction.

The reaction in immune or sensitized rabbits is not specific for the homologous species of *Leishmania*.

Transmitted October 8, 1923.

From the Departments of Zoology and of Hygiene, University of California.

LITERATURE CITED

Bandi, I.
1913. Preliminary note on the identity of certain leishmanioses based on biological reactions. Jour. Trop. Med. and Hyg., **16**, 50.

Coca, A. F.
1922. Studies on specific hypersensitiveness. Jour. Immunol., **7**, 163–178.

Coca, A. F., and Cook, R. A.
1923. On the classification of the phenomena of hypersensitiveness. Jour. Immunol., **8**, 163–182.

Delanoe, P.
1911. L'immunité naturelle de la souris à l'égard des cultures de Kala-Azaz et de bouton d'Orient tunisiens. C. R. Soc. Biol., Paris, **70**, 387–388.

Fairley, K. D.
1923. The investigation of the immunity reactions in hydatid disease. Med. Jour. Australia, **10**, II, 27–37.

Force, J. N., and Stevens, I. M.
1917. Further studies on typhoidin. Arch. Int. Med., **19**, 444–456.

Gaspari, F. de.
1921. Morbo coitale maligno e anafilassi. Alcune recerche sperimentali sulla sensibilizzazione dell'organismo animale con i prodotti tossici du trypanisomi e con le cue lorazione. Clin. Vet. Milano, **44**, 671–687.

Hamman, L., and Wolman, S.
1912. Tuberculin in diagnosis and treatment (New York, Appleton), xiv + 381 pp., 30 figs. in text.

Krause, A. K., and Peters, D.
1920. Immunity in tuberculosis. Am. Rev. Tubere., **4**, 551–556, 2 pls.

Lanfranchi, A., and Sani, L.
1921. L'intrapalpebroreazione nella diagnosi del morbo coitale maligno. Clin. Vet. Milano, **44**, 647–650, 1 pl. in text. (Trop. Dis. Bull., **19**, 150.)

Laveran, A.
1917. Leishmanioses (Paris, Masson), iii + 515 pp., 4 pls., 40 figs. in text.

Meyer, K. F., and Christiansen, O. R.
1917. The intracutaneous "typhoidin" reaction. Jour. Inf. Dis., **20**, 357–441.

Nusshag, W.
1921. Allergische Reactionen bei der Beschälseuche. Berl. tierarztl. Wochenschr., **37**, 567–569.

Von Pirquet, C.
1911. Ueber die verschiedenen Formen des allergischen Reaktion bei der Revaccination. Ztschr. f. Immunitatsforsch. u. exper. Therap., Orig., **10**, 1–23, 13 figs. in text.

EXPLANATION OF PLATE 42

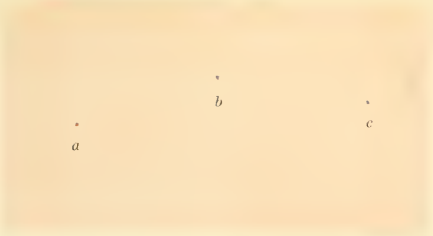
All reactions shown forty-eight hours after the intradermal injection of alkaline extracts of *Leishmania tropica* and *Leishmania infantum*. Figures natural size and color. Drawings by Dr. Olive Swezy.

Fig. 1. Normal rabbit No. 21 showing the results of the intradermal injection of a 50 per cent dilution of stock solution of the alkaline extracts of *L. tropica* and *L. infantum*. *a*. Alkaline extract of *L. tropica*. *b*. Alkaline extract of *L. infantum*. *c*. Extracting fluid control. Drawn September 24, 1923.

Fig. 2. The same as fig. 1 on immune rabbit No. 7. *a*. 50 per cent dilution of the alkaline extract of *L. tropica*. *b*. 50 per cent dilution of the alkaline extract of *L. infantum*. *c*. Extracting fluid control. Drawn September 24, 1923.

Fig. 3. Immune rabbit No. 7 showing the effect of the intradermal injection of the stock alkaline extracts of *L. tropica* and *L. infantum* as compared to a 50 per cent dilution of the stock alkaline extract of *L. tropica*. *a*. Extracting fluid control. *b*. Stock alkaline extract of *L. tropica*. *c*. 50 per cent dilution of the stock alkaline extract of *L. tropica*. *d*. Stock alkaline extract of *L. infantum*. Drawn September 17, 1923.

Fig. 4. Immune rabbit No. 127 injected intradermally with alkaline extracts of *L. tropica* and *L. infantum* containing 2,000,000 flagellates per cubic centimeter. *a*. Alkaline extract of *L. infantum*. *b*. Extracting fluid control. *c*. Alkaline extract of *L. tropica*. Drawn September 10, 1923.



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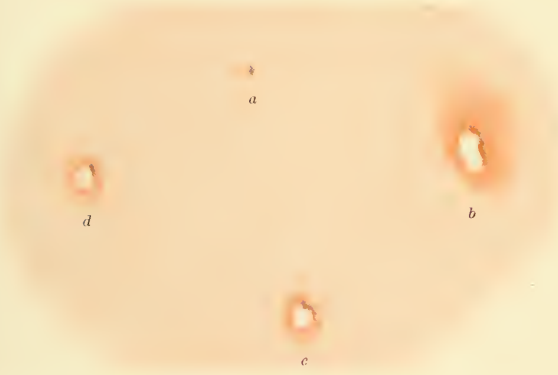

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THE DISTINGUISHING CHARACTERISTICS OF THE PARASITIC AMOEBAE OF CULTURE RATS AND MICE

BY

JOHN F. KESSEL

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CONTENTS

	PAGE
Introduction	490
Historical	490
Acknowledgments	491
Material and methods	492
Incidence of infection of amoebae in rats and mice	493
Councilmanio muris	495
The motile amoeba	495
Encystment	503
Budding	507
Mitosis	509
Councilmania decumani	511
The motile amoeba	512
Encystment	512
Budding	514
Mitosis	514
Endamoeba ratti	515
The motile amoeba	515
Encystment	516
Experiments in cross-infection	517
Discussion	522
Summary	529
Literature cited	531
Explanation of plates	534

INTRODUCTION

The present investigation was begun in order that, later, we might attempt the experimental infection of rats and mice with the common intestinal amoebae found in man. For it was early realized that, before such critical experimental infections could be carried out satisfactorily, a detailed study of the amoebae normal to the rat and mouse was essential.

Our attempts to culture in rats and mice most of the common intestinal amoebae of man have met with success (Kessel, 1923*b*), and it is our purpose in the present paper to describe the distinguishing characteristics of the amoebae normally occurring in culture rats and mice.

HISTORICAL

Grassi (1881) found in both the rat and the mouse an amoeba which he named *Amoeba muris*. He published no figures and described no cysts but stated that the motile forms resembled *Amoeba coli* (Lösch) in man. He distinguished the amoeba of the mouse from *E. coli* largely on the basis of size, and stated that the maximum size of the species found in the mouse slightly exceeds the minimum size of the species found in man.

Wenyon (1907) in connection with his work on the intestinal Protozoa of mice made a careful study of an amoeba found in the mouse, and concluded that the free forms and cysts bore a very striking resemblance to *Endamoeba coli*. When the genus *Councilmania* was later described (Kofoid and Swezy, 1921), resemblances were at once apparent between this new genus and the amoeba described by Wenyon. These are also very well shown in Wenyon's figures.

Wenyon's work is excellent as a pioneer investigation, but certain structures, such as the chromatoidal body, were not then regarded as important diagnostic characters and were described (his pl. 10, figs. 13, 20) as being remains of food products that had been thrown out of the cyst. It is now generally accepted that solid food products are extruded in the precystic stage. His account of autogamy in the amoeba of the mouse, as previously described by Schaudinn for *Endamoeba coli*, cannot be upheld in the light of our present knowledge of nuclear phenomena in cysts.

Brug (1919) concluded that the amoebae of the rats, identified as *Mus rattus*, and of the mice examined by him were identical, and that their resemblances to *Endamoeba coli* were close, the greatest difference being in the size. His figures indicate that he was dealing, at least for the most part, with the species *Councilmania decumani*. Rudovsky (1921) described and figured an amoeba found in five rats of the species *Mus decumanus* Pall., which is a synonym for *Rattus norvegicus* (Erxleben). Their figures show that Rudovsky and Brug have deseribed an identical species. Rudovsky, however, drew a morphological distinction between *Endamoeba coli* and the amoeba of the mouse, which he described as having a heavy nuclear membrane, a large karyosome, and little outer chromatin; and also, between these two amoebae and the amoeba of the rat, which he deseribed as having a thin nuclear membrane, small granules of peripheral chromatin, and a small karyosome. He speaks of this amoeba of the rat as *Endamoeba muris decumani*.

In our own work, following investigations, first of an amoeba of the mouse and then of an amoeba of the rat, it was thought that there was a difference between the morphological characteristics of the two amoebae. But a second lot of rats produced amoebae identical in all respects with the amoeba found in the mouse. For a time it seemed that the differences noted in the first rats examined represented different phases of the same life cycle, but further investigation has led to the conclusion that there are at least three species of amoebae parasitic in the intestine of culture rats and mice.

For reasons to be stated later, these three species of amoebae should be classified as *Councilmania muris* (Grassi, 1881, emend. Wenyon, 1907) Kessel emend.; the species *Councilmania decumani* (Rudovsky, 1921) Kessel, emend.; and *Endamoeba ratti*, sp. nov., a species having granular pseudopodia and found, to date, only in the rat.

ACKNOWLEDGMENTS

The writer is greatly indebted to Professor C. A. Kofoid, under whose supervision the work here presented has been completed; to Dr. E. L. Walker of the Hooper Foundation, who has offered many helpful suggestions; and to Dr. Olive Swezy, who has been generous in extending many courtesies. The work of the past year has been made possible by the writer's appointment as University Fellow in Zoology.

MATERIAL AND METHODS

Culture albino mice, *Mus musculus* Linnaeus, obtained from four different colonies, and culture rats, *Rattus norvegicus* (Erxleben), were used. The colony of rats from which our stock came was started in 1911, when Professor J. A. Long crossed three albino females, obtained from the Wistar Institute, with a wild brown male, presumably *Rattus norvegicus*, caught in Berkeley. New commercial stock of albinos has been added once to this colony. Huber (1915), quoting Donaldson, concluded that the albino rats from the rat colony of the Wistar Institute of Anatomy and Biology belong to the species, *Mus norvegicus albinus* (Donaldson). Dr. Joseph Grinnell, of the California Museum of Vertebrate Zoology, has identified an adult brown rat taken this year from our stock colony as *Rattus norvegicus*.

To determine the presence of amoebic infection in the culture animals, the normal faeces from the rats and mice were at first examined daily. This method proved to be slow, tedious, and inaccurate (see Brug, 1919, and Kessel, 1923*a*). Successful attempts were then made to evacuate the amoebae in the faeces in greater quantities by the administration of a purgative, epsom salt, as described by Kessel (1923*a*), and this method was used throughout the investigation.

Preliminary examinations were made of all material in fresh smears, one-half of the cover-glass preparation being smeared in normal salt solution, the other half in Donaldson's iodine-eosin stain. The active forms are easily detected in the normal saline solution, while the cysts are more readily seen in the stained portion. Permanent slides were made by fixing the material for two minutes in Schaudinn's fluid heated to 60° C. The slides were stained in iron-haematoxylin.

The activity of the motile forms has been observed in normal saline on an electric warming stage at 37° C.

INCIDENCE OF INFECTION OF AMOEBAE IN RATS AND MICE

During the investigation 80 mice and 288 rats have been examined for amoebae. As stained slides have been made in only 45 of the positive cases found, the exact species of amoebae has been determined only in these 45 rats and mice. The other examinations were made for the purpose of procuring animals for infection experiments and were studied only in iodine-eosin. The data in the first table serve merely to differentiate between amoebae of the genus *Councilmania* and the genus *Endamoeba*.

TABLE 1

TABLE SHOWING INCIDENCE OF INFECTION OF RATS AND MICE WITH AMOEBAE BELONGING TO THE GENERA *Councilmania* AND *Endamoeba*

Mice

Lot No.	Where Obtained	Age	Positive		Negative	Total	Per cent positive
			Councilmania	Endamoeba			
1	Dr. Long	Over 10 months	3		7	10	30.0%
2	Dr. Morgan	2 to 4 months	4		5	9	44.5%
3	Dr. Hagedoorn	2 to 4 months	7		8	15	46.6%
4	Dr. Long	About 10 months	7		11	18	39.0%
5	Dr. Morgan	Five weeks	2		7	9	22.0%
6	Dr. Long	About 10 months	2		10	12	16.6%
7	Dr. Gaylord*	2 to 4 months	5		2	7	71.2%
			30		50	80	37.5%

Rats

Lot No.	Where Obtained	Age	Positive		Negative	Total	Per cent positive
			Councilmania	Endamoeba			
1	Dr. Long	4 to 6 months	6	1	13	20	35.0%
2	Dr. Evans	Over 10 months	1		19	20	5.0%
3	Dr. Long and Dr. Evans	Mixed	29	1	77	107	28.0%
4	Stock	2 to 4 months	17		8	25	68.0%
5	Stock	2 to 4 months	23		2	25	92.0%
6	Stock	3 to 5 months	8	1	9	18	50.0%
7	Stock	2 months	6		14	20	30.0%
8	Stock	5 and 6 weeks	0		15	15	0.0%
9	Stock	5 and 6 weeks	3		15	18	16.6%
10	Stock	Over 10 months	2		18	20	10.0%
			95	3	190	288	34.0%

* New York State Institute for the Study of Malignant Diseases.

The following conclusions may be drawn from a study of the data in the accompanying tables.

1. The incidence of infection of amoebae among culture mice in this laboratory is 37.5 per cent while among rats it is 34 per cent (see table 1).

2. The incidence of infection is much lower in very old and in very young mice and rats than it is in middle-aged animals (table 2).

TABLE 2

Mice

Age	Positive	Negative	Total	Per cent positive
Under 2 months	2	7	9	22.0%
2 to 4 months	16	15	31	51.6%
About 10 months	12	28	40	30.0%

Rats

Age	Positive	Negative	Total	Per cent positive
Under 2 months	9	44	53	17.0%
2 to 10 months	54	42	96	56.3%
Over 10 months	3	40	43	7.0%

3. The incidence of infection is lower in wild mice and rats than in culture mice and rats (see table 3). It is assumed that the mice used by Wenyon were culture mice, and while he gives no figures of the number he examined he states that he found amoebae in about half of the cases. The culture mice and rats examined in this laboratory show an average infection of 35.7 per cent, while all the wild rats and mice recorded in the literature and the 20 wild mice examined in this laboratory show an average infection of 7 per cent.

Balfour (1922) made his examinations from autopsied rats and it is assumed that Rudovsky worked with dead animals. The examinations of the wild mice in this laboratory were made after death. Examination after autopsy should be just as certain a method of determining infection as the epsom salt method. Brug does not state how his 50 rats were examined, but, as his rate of infection is higher than Rudovsky's and only slightly lower than the lower percentage recorded by Balfour, it would seem fair to include his results in the table. These results indicate that animals living in the crowded conditions of a laboratory colony are more liable to acquire amoebic infection than those living in the open.

COUNCILMANIA MURIS (Grassi, 1881), emend. Kessel

Occurrence.—Councilmania muris has been found in 14 of the 25 rats and in 4 of the 17 mice from which stained slides of the faecal material have been prepared in this laboratory (see table 4). An examination of the stained slides has been our main and most conclusive method of differentiating this species from *Councilmania decumani;* the positive cases diagnosed in fresh smears only have not been considered in the percentages cited above.

TABLE 3

Table Showing Incidence of Amoebic Infection in Rats and Mice Recorded in Literature

Investigator	Species of Animal	Positive	Negative	Total	Per cent positive
Wenyon	Mus musculus	Not stated	Not stated	Not stated	50.0%
Brug	Mus rattus	7	43	50	14.0%
Rudovsky	Mus musculus	0	37	37	0.0%
Rudovsky	Mus musculus	5	159	164	3.0%
Balfour	Black rats	Not stated	Not stated	34	25.0%
Balfour	Brown rats	Not stated	Not stated	444	15.7%
Kessel	Mus norvegicus	96	172	268	3.6%
Kessel	Mus musculus (albino)	30	00	80	37.5%
Kessel	Mus musculus (wild)	2	21	23	9.0%

TABLE 4

Incidence of Infection of Amoebae of Rats and Mice Determined from Examination of Slides Stained with Iron-Haematoxylin

	Councilmania muris		Councilmania decumani		Endamoeba ratti		Mixed per cent C. muris and C. decumani	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Mice	4	23	14	70	0	0	2	12
Rats	14	56	8	32	2	8	1	4

The Motile Amoeba

Habitat.—The normal habitat of this amoeba is the caecum. Unless examination is made following a purgative, the motile forms are seldom found in the colon at autopsy. Just how the active amoebae are restricted in habitat almost entirely to the caecum, while the contents of the caecum within which the amoebae have been mixed are discharged into the colon, affords an interesting problem. According to Cannon and McNease (1923) the pH of the caecal contents is more

acid than the contents of the ileum or the colon. Determinations of the pH by us in this experiment verify this conclusion and it would seem that a definite chemotropism restricts the active amoebae to this region.

The degree of infection varies greatly; in some instances as many as three or four amoebae have been found in a single field of a freshly prepared smear, while in other cases only a single amoeba has been found in an entire smear. There is no evidence that the degree of infection varies in different parts of the caecum, for if amoebae are present in a smear from one region they are usually present in other parts of the caecum. They may be scraped with caecal contents from the surface of the epithelium, but no visible pathological conditions have been observed in the wall of the caecum.

Food and pathogenicity.—The amoebae live normally upon food procured from the contents of the caecum. They may ingest bacteria (pl. 43, figs. 1, 2), yeasts, and flagellates (pl. 43, fig. 4), as shown by the presence of a partly digested *Chilomastix* in the largest vacuole. At the suggestion of Dr. I. C. Hall of the Department of Bacteriology in the University of California, gram stains were made of the free amoebae from the caecum in order to determine the type of bacteria ingested. After a number of attempts, successful results were obtained. Bismark brown was found to be the most successful counterstain. The cytoplasm is gram-negative while the nucleus retains the gentian violet. The amoebae ingest both gram-positive and gram-negative bacteria. the cases examined giving 75 per cent gram-negative rods and 25 per cent gram-positive cocci. It is significant that no gram-positive rods have been found ingested, though many are present on the slides in the material surrounding the amoebae. Whether or not a change in the stainability of the bacteria is produced by their partial assimilation by the amoebae which causes them to stain gram-negative, it is as yet impossible to say. *Sphaerita* (Dobell, 1921) has been found as a parasite in the amoebae (pl. 43, fig. 1).

Because no evidence has been encountered which indicates that the free amoebae ingest normal tissue cells—the reason being that no caecal ulcers have been found in any of the rats—and because 34 per cent of normal rats harbor amoebae in the caecum, it is thought that no pathogenic results accompany the presence of these amoebae in the rodent hosts.

Pseudopodia and amoeboid movement.—As examination in normal saline reveals no marked specific differences between *Councilmania*

muris and *Councilmania decumani*, the pseudopodia and the amoeboid movement of the two species will be discussed together.

Grassi (1881) in describing the amoebae of the rat and mouse said that they are slightly motile and that two or more large and blunt pseudopodia can be demonstrated. Ordinarily, only one is evident at a single instant and this is retracted before the second is formed. He described the pseudopodia as "hyaline," i.e., ectoplasmic, and stated that the endoplasm flowed into the ectoplasm, which he interpreted as the method by which the amoeba moves.

Movement, according to Wenyon (1907), may be rapid on a warm stage. He wrote, "As a rule only a single pseudopodium is formed at one time. This consists at first only of ectoplasm [his pl. 10, fig. 3] into which the endoplasm suddenly streams, carrying the nucleus with it." Brug (1919) stated that the amoebae creep about more actively than he ever saw *Endamoeba coli* creep and that in the pseudopodia only ectoplasm is found.

Rudovsky (1921) found that the protruding pseudopodia are plainly divisible into a transparent and a finely granular zone and he differentiates between ectoplasm and endoplasm (his pl. 7, fig. 1). It is evident from these descriptions that the amoebae already described from rats and mice possess hyaline pseudopodia.

Endamoeba coli (Lösch) from man, on the other hand, differs remarkably from these amoebae in this regard. Dobell and O'Connor (1921) stated that in the change of shape of *Endamoeba coli*, without evident progression (fig. on their pl. 1), "no sharp line of demarcation separates the ectoplasm and endoplasm, and the formation of large, clear, blade-like pseudopodia—so characteristic of *Endamoeba histolytica*—is never seen."

One of the distinguishing characteristics between *Endamoeba coli* and *Councilmania lafleuri* (Kofoid and Swezy, 1921) is this difference in formation of pseudopodia, hyaline pseudopodia being the type possessed by *Councilmania lafleuri*. It therefore seems quite evident that in this one respect alone there is sufficient differentiation to warrant drawing a distinction between *Endamoeba coli* and the amoebae of the rat and mouse.

The formation of pseudopodia of *Councilmania muris* and of *Councilmania decumani* can be described most satisfactorily by division of their movements into two types: first, that during progressive movement and second, that during attachment to the substrate. In both types of movement the pseudopodia are characteristically hyaline.

It is not always possible to procure amoebae, even from the caecum, that will remain active for more than a few minutes on the warm stage. At times, however, amoebae have been secured which have exhibited typical movement for thirty minutes. The reasons for this variation in activity are undetermined.

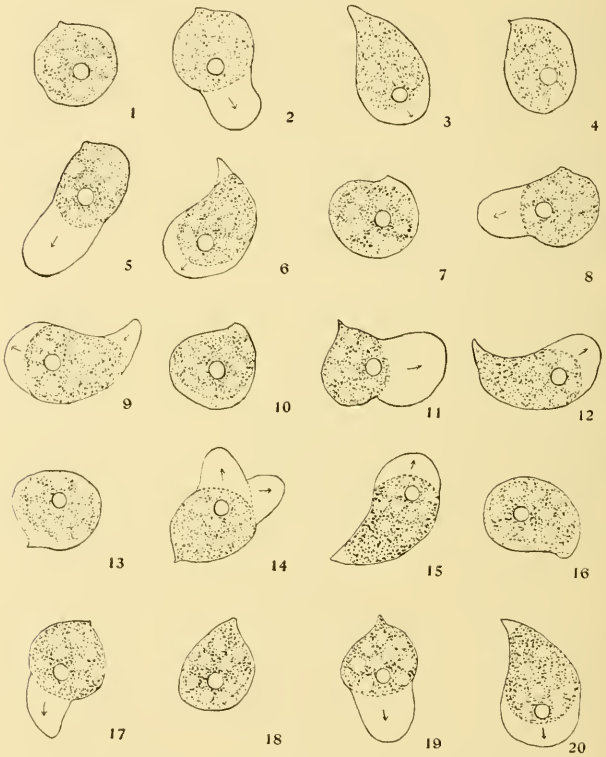

Fig. A

Free-hand sketches, showing progressive movement, of *Councilmania muris* from rat.

In progressive movement an amoeba may move as far as 500 microns in five minutes. Usually, however, the movement is less rapid.

This forward movement may be described as an ectoplasmic looping. As represented in figure A, 1, the rounded amoeba shows at the most only a narrow ectoplasmic margin. This rounded amoeba protrudes, with almost explosive suddenness, a hyaline pseudopodium (fig. A, 2) into which the endoplasm may later flow (fig. A, 3). In the progressive movement, the pseudopodia appear blade-like in optical section, but in reality are blunt lobes. They are inclined to be more pointed than the pseudopodia formed when the amoeba is attached. In the forward movement, the hyaline pseudopodium is fully formed before the vacuolated and granular endoplasm flows into it. At the posterior, or attached end, a conical projection or attachment cone is often in evidence (fig. A, 1). This appears to be used as a point of adhesion to the substrate and as a point of leverage. As the endoplasm flows forward, this root-like projection persists for a short time as a hyaline structure (pl. 43, fig. 3). The amoeba then resumes a spherical or ovoidal shape before it thrusts out another pseudopodium. The whole process is then repeated.

In progressive movement the pseudopodia are not all thrust out in a constant forward direction, but lateral pseudopodia (figs. A, 8, 11) or even posterior pseudopodia (fig. A, 14) may be formed. In the observations made, however, a permanency of movement in a given direction was maintained. The formation of these lateral and posterior pseudopodia may indicate a reversing motor reaction such as is found in ciliates, or it may indicate that the path, if observed for a sufficient time, might represent a "flattened spiral" as described by Schaeffer (1920). As in these amoebae there appears to be no constancy in the formation of left or right-handed pseudopodia, it is not certain that either a right or left-handed spiral would be retained. In progressive movement, it is not common to see two pseudopodia (fig. A, 14) in action at the same time, and this probably occurs only when the amoeba changes its direction, as perhaps in a motor reaction.

Pseudopodial formation during attachment. — In a cover-class preparation, the amoeba often appear to be attached either to the slide or to the cover-glass, as they may also adhere to a clump of bacteria from which they are apparently making a continuous effort to become free. Pseudopodia are then thrust out rapidly in all directions, occasionally as many as three or four being evident at one time (fig. B, 11; pl. 43, figs. 1, 2). These pseudopodia are always hyaline and while some present the pointed, blade-like appearance of the one formed by amoebae in progressive movement (fig. B, 12), the broad, blunt,

balloon-like pseudopodia (pl. 43, fig. 2; fig. B, 1) are more common. The endoplasm may move into and fill the pseudopodia formed when the amoeba is thus attached, but more commonly the pseudopodia are retracted or a pseudopodium may be partly filled with endoplasm and the remaining portion retracted.

In accordance with the gel-sol theory of pseudopodial formation (Hyman, 1917), this hyaline ectoplasm after protrusion represents a gel and the more vacuolated endoplasm a sol, each of which is reversible, one into the other, as protrusion, advancement, or retraction occurs.

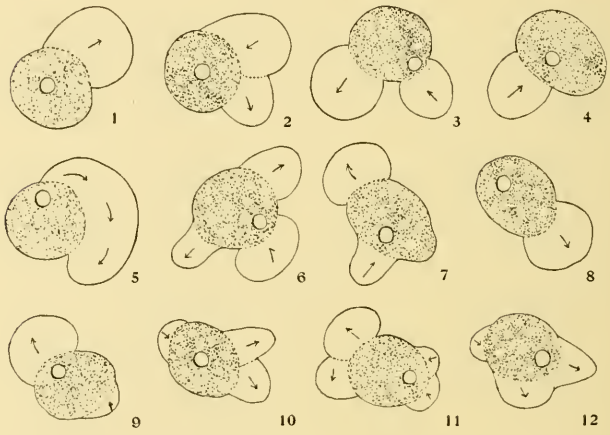

Fig. B

Free-hand sketch, showing formation, while attached, of pseudopodia of *Councilmania muris* from rat.

In the formation of a pseudopodium, solution of the ectoplasm occurs at the initial point of formation. This liquid sol spreads outward and forward, its outer surfaces forming a gel, as contact occurs with the medium of the environment. The liquid endoplasm then flows in and occupies the space between these gelated layers. In retracting a pseudopodium, the gelated ectoplasm is gradually reversed to a sol state.

In this discussion it is preferred to describe the protoplasm as separated into two layers, the ectoplasm and endoplasm, rather than to

describe a third outer layer, the hyaloplasm, as is found in some amoebae (see Schaeffer, 1920). While it appears at times that there are two layers outside the endoplasm, a thin, clear outer layer and an alveolar middle layer, these two layers blend so, one into the other, that, as a rule, the line of demarcation is indistinguishable. These two layers will therefore be described as constituting the ectoplasm. In stained slides the ectoplasm appears to be finely alveolar in structure.

The great activity with which the amoebae move forward, the remarkable coördination during the progressive movement, the suddenness with which pseudopodia are formed, the contractility exhibited by the protoplasm, and the indescribable contortions indulged in by the active amoebae, lead one to disregard the earlier theories, which endeavored to explain amoeboid movement as the result of surface tension, and to conclude with Schaeffer (1921) that the mechanism controlling locomotion and movement is primarily internal rather than that the pseudopodia move the amoeba.

The endoplasm contains the nucleus and the numerous vacuoles, some containing undigested food and some being filled with liquid. The endoplasm which surrounds these structures presents a flaky appearance similar to that of the cytoplasm which is found in the encysted stages. No contractile vacuole has been observed at any time.

Size.—Grassi (1881) described the average size of the motile amoebae as 13.2μ. Wenyon (1907), however, recorded the motile forms as measuring from 30–40μ. Rudovsky (1921) figured motile forms measuring 30μ in diameter. The rounded motile forms, measured on permanently stained slides in this investigation, average 19μ. With extended pseudopodia, the length is greatly increased, forms 40μ in length and 30μ in breadth having been encountered. An attempt to determine racial differences in size within the species, from the motile forms, such as is very evident among the cysts, indicates that there is a corresponding racial difference in the motile forms, the average diameter of the largest race in this phase being 21.5μ while the average of the smallest is 18.1μ. The cysts of these races average respectively 17.7μ and 14.5μ. This affords an approximate ratio of the volume of rounded unencysted amoeba to the cyst as 1.2 is to 1; i.e., the volume of the rounded motile amoeba is 0.2 greater than the corresponding cyst which it forms.

Nuclei.—The nuclei of free forms of *Councilmania muris* are distinguishable from the food vacuoles in normal saline solution by a more opaque appearance and by a darker gray color. In the iodine-

eosin stain the nuclei take the iodine stain first and for a brief period it is possible to definitely distinguish the structure; but as the amoeba gradually assumes the red color of the eosin and disintegrates, the well-defined nuclear structure disappears. In the slides stained with iron-haematoxylin, the same distinguishing characteristics are shown that are found in the nuclei of encysted stages. The nuclear membrane is very thin and stains very faintly (pl. 43, fig. 3). A vacuole surrounding the nucleus has been figured by Wenyon (1907, pl. 10, fig. 3). This condition has been seen in my own investigations, but I have considered it to be an abnormality resulting from staining rather than as a normal occurrence.

Little or no chromatin is encrusted on the nuclear membrane (pl. 43, figs. 1 and 3). This condition, in which small chromatin granules are found lining the internal margin of the nuclear membrane, is characteristic of a very early prophase. The bulk of the chromatin material within the nucleus occurs in a 'central' karyosome of the dispersed type, in the form of clustered spherical granules *which always present a more or less dispersed appearance*. At times the granules may be congregated in a dispersed central mass (pl. 43, fig. 4). This dispersed type of karyosome is figured by Wenyon (his pl. 10, figs. 1–3) and while he notes a distinct nucleolus (his pl. 10, fig. 1) this is shown as being more dispersed than is the case in *C. decumani*. A network of faint linin fibers connects the chromatin granules with the nuclear membrane. The average diameter of the nuclei in motile forms is 5.6μ.

The nuclei do not retain a constant spherical form but are elastic and may be elongated at times. In one instance the nucleus in a living amoeba elongated and constricted so definitely in the middle that it was thought division was in progress. The nucleus, however, soon resumed its normal spherical shape and did not divide. It was kept under careful observation until the amoeba had again assumed a rounded condition.

Asexual reproduction.—It is assumed that this species of amoeba multiplies by two methods, by binary fission of the motile amoeba and by budding, a modification of multiple fission following encystment. No occurrences of binary fission have been witnessed in the motile stages of this species though motile stages of *C. decumani* have been found in which two nuclei were present (pl. 46, fig. 31) and the last stages of a division process were seen in a case of *C. lafleuri* which had been recovered from a case of infection established in a rat (see Kofoid, Swezy, and Kessel, 1923*b*).

Precystic stage.—In the motile stages of this amoeba, a certain number of the vacuoles usually contain solid food material. As the amoeba prepares for encystment, all solid material is either digested or egested, leaving the vacuoles filled with liquid substance only. These vacuoles tend to coalesce, and a few large vacuoles result from a number of small ones (pl. 43, fig. 5).

The amoeba assumes a spherical form and in time secretes a cyst wall (pl. 43, fig. 7). There is a further coalescing of the vacuoles at about this stage (fig. 6), until a single large vacuole is formed in the early phase of encystment (fig. 7). This vacuole has the subcentral position of the glycogen mass, the glycogen having been dissolved out in the process of staining. It seems certain, however, that this glycogen material is derived, at least in part, from the smaller vacuoles found in the precystic stages that contain liquid. There is an analogy between this method of formation of vacuoles and the method of the formation of contractile vacuoles in ciliates as described by Taylor (1923).

Encystment

Occurrence.—Until recently it was generally considered that encystment was for the purpose of protecting the organism while it was being transferred from host to host. There seems, however, to be growing evidence that encystment may occur periodically, following intervals of the accumulation of excess food material (Kofoid, 1923). It has been noted that, in cases where the cysts appear in the faecal material, they also usually occur in the caecum, indicating that encystment, for the most part, takes place in the caecum. It is further significant to note that following the administration of epsom salt there is no regular response in the formation of cysts. One would think that, as a result of exposure to this deleterious environment, the forms would encyst immediately. This does happen occasionally and a few hours after the extrusion of the amoebae has begun, cysts may be found in the faeces. It happens just as often, however, that no cysts appear in the faeces, although motile amoebae may appear for a period of two or three days or until the final effects of the purgative have disappeared. Numerous cysts are often encountered on the first examination after the purge is administered, indicating that the cysts were already formed in the caecum, and that the caecal contents, whether they contain free amoebae, encysted forms, or a mixture of the two, are emptied to the outside.

A daily examination of the normal faeces, as they may be pressed from the rectum of the rat, shows that there is no regular periodicity in the formation of cysts (Brug, 1919, and Kessel, 1923*a*). At times as long an interval as three weeks may elapse between the appearance of cysts in the faeces, while at other times they may appear daily for three consecutive days.

It is possible to detect the cysts in normal saline solution by their light gray color and by the inner darker layer of the peripheral wall which surrounds the cyst. It is, however, extremely difficult to distinguish the nuclei, and differentiation of species should not be based on this type of superficial examination alone. In this preparation the glycogen mass appears as a large fluid-filled vacuole.

In the iodine-eosin stain the cysts assume a greenish-yellow color, presenting a shade which is about midway between the pale yellowish green cysts of *Endamoeba dysenteriae*, and the more yellow cysts of *Endamoeba coli*. In this preparation the nuclei stain definitely, and the type of karyosome often may be clearly demonstrated. The glycogen masses which are present in a certain number of the cysts are always stained dark brown by the iodine.

Shape.—The majority of the cysts are spherical or ellipsoidal in shape, in optical section appearing round (pl. 45, fig. 23), perfectly elliptical (pl. 45, fig. 20), or presenting varying degrees of elliptical outline (pl. 43, fig. 7; pl. 44, fig. 10). No extremely irregular cysts, like those often encountered in *C. decumani* (pl. 47, fig. 49), have ever been observed by us in *C. muris*.

Cyst wall.—A thin wall surrounds the cyst. In the iron-haematoxylin stain this appears quite transparent and can be defined only by the inner and outer margins. In the iodine-eosin preparations, however, it appears as a circle composed of three layers, in optical section consisting of a thin outer line, a middle clear zone, and an inner dark line. This resembles the cyst wall of *C. lafleuri* (Kofoid and Swezy, 1921). The thickness of the cyst wall is relatively constant in all cysts, no matter what their dimensions may be. Measurements show only a slight variation from 0.50μ in the newly-formed cysts to 0.75μ in the larger and older cysts.

Sizes and races of Councilmania muris.—Wenyon and O'Connor (1917) and Dobell and Jepps (1918) have shown that *E. dysenteriae* and *E. coli* are divided into a number of distinct races, the determination being based on the size of the cysts. The five races of *E. dysenteriae* have mean diameters of 6.6, 8.3, 11.6, 13.3, and 15μ respectively,

while in *E. coli*, the four races distinguished have mean diameters of 15, 16.5, 18.7, and 21.7μ respectively.

In *C. muris* the cysts vary in diameter from 13μ (pl. 44, fig. 16) to 19μ (pl. 45, fig. 23). Measurements of cysts have been made from the material stained in iron-haematoxylin and three distinct races have been determined, one with an average diameter of 14.5μ, a second with an average of 15.7μ, and a third with an average of 17.7μ. The accompanying table gives the range, average diameter, standard deviation, coefficient of dispersion, and variability in percent of the different races of *C. muris*. Cross-infection experiments of *Councilmania muris* to non-infected rats have produced no racial change in the size of the cysts.

TABLE 5

	Range	Races, Average diameter	Standard deviation	Coefficient of dispersion	Variability in per cent
C. muris.	13 to 16	14 5	.92	.0634	.44%
	15 to 17	15 7	.68	.0511	.33%
	16 to 21	17 7	1 51	.0856	.43%

Cytoplasm and inclusions.—The cytoplasm in the cysts of *C. muris* in structure looks like an accumulation of fine flakes. Stained with iron-haematoxylin it assumes a gray appearance and the darker inclusions within the cyst may be distinguished easily. This flaky structure of the cytoplasm can be distinguished definitely from the alveolar type of cytoplasm found in *E. dysenteriae* and from the more coarsely granular cytoplasm of *E. coli*.

Three types of structures within the cytoplasm of the cyst are to be distinguished, namely, the nuclei, and two types of inclusions, the glycogen mass and the chromatoidal bodies.

The glycogen mass, though present in the cysts at the time of fixation, is dissolved out by water during the process of staining and leaves merely a large clear vacuole. This vacuole is predominantly present in the mononucleate and binucleate cysts (pl. 43, fig. 7; pl. 44, fig. 11). An occasional mononucleate cyst is found without a glycogen vacuole (pl. 44, fig. 9), but it is significant to note that no binucleate cysts without glycogen vacuoles have been discovered. As the glycogen is probably utilized in the metabolic processes attendant upon mitosis, it seems likely that a mononucleate cyst without glycogen cannot complete development. Around the margin of the glycogen vacuole chromatoidal bodies often appear (pl. 44, fig. 15). As the chromatoidal

bodies often appear coincidently with the disappearance of the glycogen mass, it seems likely that the latter may function in the formation of the former (Kofoid, 1923). These may be used up early in the developmental process as is evidenced by the fact that many of the four-nucleate, five-nucleate, and eight-nucleate cysts do not possess chromatoidals (pl. 44, figs. 17–19) or they may persist to the eight-nucleate stage (pl. 45, fig. 21). These chromatoidal bodies in the latter stages have the form of massed bundles of splinter-like processes as is shown by the irregular ends of the masses (figs. 24, 27). It is likely that these massed chromatin bodies are formed by a union of the smaller bodies that first appear around the margin of the glycogen mass as is described for *C. lafleuri* (Kofoid and Swezy, 1921). The scattered acicular and filamentous type of chromatoidal body so often seen in *E. coli* has not been found in this species.

Four-nucleate cysts may be found either with or without the glycogen vacuole (pl. 44, figs. 16–17) though it is quite unusual to find the vacuole persisting longer than to the four-nucleate stage.

Nuclear phases.—As the nuclei within the cysts are frequently in a state of mitotic change, it is necessary to regard these changes in making a diagnosis. Nuclei in cysts, prior to the eight-nucleate stage, may show figures representing any phase of mitotic development. The typical resting nucleus is more often encountered in the eight-nucleate cysts and on this account they are more commonly used as a basis of diagnosis.

The nuclear membrane of *C. muris* is characterized by its very thin and transparent appearance. Small chromatin granules may lie around the inner margin of the membrane, but this is common only in the early prophase. As a rule the nucleus is characteristically clear, and in the middle is the dispersed karyosome (pl. 45, figs. 21, 24, 25). Linin fibers connecting the karyosome with the nuclear membrane are more common in the earlier than in the later divisions. The nuclear membrane is so faint that it is frequently difficult to detect, and its position can often be determined only by the limits of the cytoplasm which surrounds the nucleus. This nucleus presents an entirely different picture from the nucleus of *E. coli*, which has a thick membrane heavily encrusted with chromatin granules, and from the nucleus of *C. decumani* the more distinct membrane of which is usually encrusted with several heavy chromatin blobs.

The nuclei in the mononucleate cysts (pl. 43, fig. 7) are the largest, and a corresponding diminution in the size of the nuclei is seen as the

number of nuclei increases. For a ratio of the size of the nuclei in this species and in *C. decumani* see Kofoid, Swezy, and Kessel (1923*a*). In material obtained after the administration of a purgative and from autopsies, the eight-nucleate cysts are the most numerous while the binucleate cysts are next in number. The binucleate cysts usually show some form of mitotic spindle and it is from this type of cyst that the majority of the chromosome counts have been made.

No sixteen-nucleate cysts have been found from this species, though nuclei have been found in the eight-nucleate cysts which indicate that a further division is in progress.

Chromophile ridge.—In a majority of the eight-nucleate cysts, a dark-staining ridge is found, which is located in the cytoplasm along the inner margin of the cyst wall. It may often extend a distance ranging from one-quarter to one-half of the circumference of the cyst (pl. 45, figs. 20, 25). This ridge is not to be confused with the chromatoidal bodies, which are usually not curved and as a rule lie embedded in the cytoplasm. Similar ridges, which they have termed chromophile ridges, have previously been described in *Councilmania lafleuri* by Kofoid and Swezy (1921). It is significant to note that Wenyon (1907, pl. 10, fig. 1) has figured a cyst which presents this typical structure.

Budding

Although it is a fact that the motile amoebae divide by simple fission, it is to be noted that this process is comparatively rare, few mitotic spindles ever having been recorded in motile forms and only a few instances of binucleate motile forms ever having been seen. The present investigation verifies this rarity of dividing motile forms, even in material collected from the caecum of autopsied animals. However, in these same caeca numerous cysts have been found, a fact which indicates that multiplication by encystment in this species of amoeba may be much more common than multiplication by binary fission of the motile phase.

The determination of the manner by which the amoebae escape from the cysts has presented numerous difficulties and has called forth several interesting theories. In *E. dysenteriae* it is generally thought that in some manner the four-nucleate amoeba escapes from the cyst, and that subsequent division into four amoebulae occurs (Chatton, 1917; Dobell and O'Connor, 1921). Again, *E. coli* is supposed to escape from the cyst in a similar manner as an eight-nucleate amoeba

(Yoshida, 1918). Wilson (1916) described pores in the cyst wall of the soil amoeba and observed the process of an amoeba emerging from the cyst through one of these pores. Kofoid and Swezy (1921) recorded the process of budding for *Councilmania lafleuri*. By this process a small amount of cytoplasm, together with a single nucleus, emerges through a pore in the cyst wall, and the cytoplasm containing the nucleus breaks off forming a small amoebula. This process is repeated until the cyst is emptied of its contents. If pores are easily demonstrable in cysts of soil amoebae, it seems likely that they may occur in other amoebae.

The present investigation of the amoebae of rats and mice was just being started at the time the observations on budding in *C. lafleuri* were recorded. Many similarities were noticed between *C. lafleuri* and the amoebae of the rat and mouse. These, together with certain figures by Wenyon (1907, pl. 10, figs. 12, 13, 18, 20, 21), though explained by him in a different manner, led to the thought that budding would probably be found in *Councilmania* of rats and mice. The assumption was easily verified by observation in the first few animals examined and numerous instances of the process have since been recorded. In *C. muris*, chromophile ridges and also chromatoidal bodies are common, and observations to date indicate that the budding process occurs in a manner similar to that in which it occurs in *C. lafleuri* (Kofoid and Swezy, 1921).

Evidence for this process is based on the following observations:

1. Buds have been seen in fresh smears prepared in iodine-eosin and normal saline, without pressure or other mechanical disturbance having been brought to bear on the cysts.

2. Buds have been seen in slides permanently stained with iron-haematoxylin. These cysts contain varying numbers of nuclei, indicating that some of the nuclei have passed out in previously formed buds. In plate 45, figure 26, the whole eight nuclei are present, one having passed into the bud; plate 44, figure 28, shows a cyst with six nuclei, one in the bud, and figure 22 of the same plate shows four nuclei in the cyst, and one in the bud. It further shows light patches in the cytoplasm, indicating spaces from which the nuclei and their surrounding protoplasm have emerged.

3. Small amoebulae about the size of buds are often found in proximity to the cysts (pl. 45, fig. 22), indicating that they have previously emerged from the cyst.

Mitosis

Accurate cytological interpretation of cell phenomena in parasitic amoebae is difficult at best and requires the careful and detailed study of a great number of specimens. In the present investigation, 170 drawings have been made with the aid of the camera-lucida and large numbers of other forms have been studied in detail with the best magnification available. The 62 figures on the plates have been chosen as the most representative of the various phases of cytological development.

The developmental process in this species of amoeba is similar in method to that recorded for other parasitic amoebae, and except in specific differences the mitotic stages simulate those already described for *C. lafleuri* (Kofoid and Swezy, 1921) and for *Endamoeba coli* (Swezy, 1922). The nucleus in the mononucleate cyst divides mitotically to form two nuclei, a second division then occurs to form four nuclei, and finally a third division, which results in a typical eight-nucleate cyst which is regarded as the mature cyst of this species.

The typical resting stage of the nucleus has already been described. This persists for a longer or a shorter time between each division, is always typical, and is found most commonly in the eight-nucleate cysts. The second division seems to require a longer time than either of the following divisions, judging from the fact that mitotic figures are most commonly encountered in binucleate cysts. Owing to the presence of the glycogen vacuoles, the nuclei are usually somewhat flattened and pressed to one side of the cyst (pl. 44, fig. 11).

Prophase.—The fact that more prophase stages were found than stages of any other phase indicates that this period occupies a longer time than any other phase of mitosis. During this stage there is a complete reorganization and transformation of the contents of the nucleus. The first indication of this phase is the arrangement of a few small granules of chromatin on the nuclear membrane (pl. 43, fig. 4). The granules of the karyosome become somewhat more dispersed than usual and numerous linin fibers connect the granules of the karyosome with the chromatin granules on the nuclear membrane. The granules on the membrane disappear and a darkly staining region appears around the granules arranged in the middle of the cyst (pl. 43, fig. 7; pl. 44, fig. 11). About this time a distinct line, the intradesmose (Kofoid and Swezy, 1921) appears between two of these granules (pl. 43, fig. 6). These granules then pull further and further apart

(fig. 7) and finally take their positions at the ends of the spindle, forming the polar caps or centrosomes (pl. 44, fig. 9). They are still connected by the darkly staining line which appears to lie in close proximity to the inner face of the nuclear membrane. During the time that the centrosomes are taking their position at either margin of the nuclear membrane, the granules of the dispersed karyosome and the dark gray region around them undergo a complete readjustment, out of which emerges a characteristic spindle upon which are arranged the chromosomes in a late prophase (fig. 8). During this process the linin network disappears and the nucleus becomes elongated (fig. 9). Wenyon (1907) stated that there did not appear to be a formation of definite chromosomes, but from the present study it appears that six chromatin masses are arranged on the spindle (pl. 44, fig. 8), and the conclusion is that these are the chromosomes. *C. muris* thus differs from *C. lafleuri*, which has eight chromosomes (Kofoid and Swezy, 1921), but agrees with *E. coli*, which also has six (Swezy, 1922).

Metaphase.—The separation of the chromosomes on the spindle to form the metaphase is not always synchronous. In plate 44, figure 9, the two upper chromosomes appear in the metaphase, while the lower chromosomes have already divided and the daughter chromosomes are beginning to separate. This non-synchronous division of chromosomes is also illustrated in the lower nucleus (pl. 44, fig. 14) in which some of the daughter chromosomes have nearly reached the polar caps, while two are still in the middle of the spindle.

The chromosomes appear as small granules, some representing spheres, while the others are somewhat elongated. There is evidence that of the six chromosomes in this species, two are large ellipsoids, two are small spheres, and the other two are slightly elongated and midway between the other pair in size (pl. 44, figs. 12, 17).

Anaphase.—After the division of the chromosomes in the metaphase there is an immediate pulling apart of the daughter chromosomes toward either end of the spindle as is shown in the lower nucleus (pl. 44, fig. 13) and the upper nucleus (fig. 14). This separation is accompanied by a disappearance of the spindle fibers between the separating daughter chromosomes and by a further elongation of the nucleus (pl. 43, fig. 13, lower nucleus). The intradesmose also becomes fainter in the median region and a complete separation of the polar caps ensues as the intradesmose retracts or fades away.

Telophase.—As good figures of this phase have been particularly rare, it is concluded that the final separation into two nuclei is com-

pleted rather suddenly. The middle nucleus of figure 18, plate 44, represents the polar cap and chromosomes arranged at either end of the nucleus, only traces of the extreme ends of the spindle fibers remaining, and shows a very marked constriction of the nuclear membrane in the middle region. This constriction of the nuclear membrane is also shown in figure 10, plate 44, but the nuclear contents have already become arranged in an early prophase of the second division. The upper nucleus (pl. 44, fig. 16), shows the final dividing stage of the nucleus into the two daughter nuclei, the nuclear contents representing a typical resting stage while the lower nucleus of the same figure shows the final separation as having occurred and the nuclear membrane fully formed around each daughter nucleus.

The process of nuclear division in parasitic amoebae differs from that in the metazoan cell in that the nuclear membrane remains intact throughout the whole process. It is for this reason that the polar caps of the spindle and the intradesmose connecting them must be formed from material within the nucleus.

The divisions of the nuclei within the same cyst do not always take place concurrently (pl. 44, fig. 15). Here the larger nucleus represents the first division while the two smaller nuclei have already completed the second division. The nuclei in figure 18 exhibit the same phenomenon: the two larger nuclei have resulted from the second division, the middle nucleus is just completing the third division, and the two small nuclei have already completed the third division.

COUNCILMANIA DECUMANI (Rudovsky, 1921) emend. Kessel

Councilmania decumani has been found in 14 of the 20 mice and in 9 of the 24 rats examined by us. We believe this is the species of amoeba from rats dealt with, for the most part, at least, in the accounts by Brug (1919) and by Rudovsky (1921). Rudovsky (1921) noted a difference between the amoeba of the mouse and the amoeba of the rat. He called the latter *Endamoeba muris decumani*. On account of the characteristics to be mentioned in the following account, it is necessary to transfer this species from the genus *Endamoeba* to which it was assigned by Rudovsky to the genus *Councilmania* (Kofoid and Swezy, 1921).

The Motile Amoeba

When examined in smears prepared in normal saline solution, there is little or no apparent difference between the motile forms of *C. muris* and *C. decumani.* The same type of hyaline pseudopodial formation (pl. 46, fig. 32) and of amoeboid movement are exhibited and the amoebae inhabit the same parts of the digestive tract. The food is similar and there is no evidence of a pathogenic effect upon the rodent host. Cases of apparent cannibalism, however, have been encountered in this species (pl. 46, fig. 30). A typical small amoeba of this species is contained in a food vacuole within the larger amoeba. The nucleus and food vacuoles of the ingested form are intact. *Endamoeba coli* has been shown to ingest cysts of *E. dysenteriae* (Wenyon and O'Connor, 1917) and Lepage (1922) recorded a very interesting instance of cannibalism in *Amoeba vespertilio* (Penard). In *C. decumani* instances of binucleate motile forms have also been encountered (pl. 46, fig. 31) by us in which the nuclei appear quite normal.

When stained in iron-haematoxylin the nuclei of *C. decumani* present an entirely different appearance from those of *C. muris.* The nuclear membrane is heavier and more distinct and much more chromatin material is encrusted thereon in *C. decumani* than in *C. muris.* In the former species, a distinct, more or less massed karyosome is found in an excentric position. This may assume the form of a sphere (pl. 46, fig. 30) or it may appear in the form of a crescent (fig. 32). The karyosome may be surrounded by a light gray halo (figs. 30, 32), or it may appear as an outstanding black sphere (fig. 31, upper nucleus). Linin fibers connect the karyosome with the chromatin material encrusted on the nuclear membrane, and small dots often appear where these fibers cross.

Encystment

The process of encystment is a generalized one for parasitic amoebae and nothing of importance differentiates the methods in *C. decumani* and *C. muris.* Of great importance, however, is the difference in appearance of the contents of the cysts. The cytoplasm in the cysts of *C. decumani* is more coarsely flaked than in *C. muris*, and the chromatoidal bodies, while often arranged in sheaves of splinter-like processes, as in *C. muris* (pl. 47, fig. 48), also commonly occur with rounded ends, curved surfaces and as small spheres (fig. 50). Chro-

matoidal bodies are not found as commonly in the cyst of *C. decumani* as in those of *C. muris*. Chromophile ridges (pl. 48, fig. 51) are not common in this species, traces of small ones having been found only three times.

The structure of the nuclei is the important feature differentiating the cysts of *C. decumani* from those of *C. muris*. As already noted in the motile forms, the nuclear membrane is more distinct in *C. decumani* than in *C. muris*, and small granules or large blobs of chromatin material are encrusted on the inner surface. This presence of characteristic chromatin material on the nuclear membrane is a constant feature throughout all the stages of mitosis. The typical resting nucleus is characterized by the presence of an excentric karyosome which is much more massed than the karyosome of *C. muris* (pl. 48,

TABLE 6

Range	Races, Average diameter	Standard deviation	Coëfficient of dispersion	Variability in per cent
C. decumani 12 to 15	14 0	1 00	.0714	.51%
14 to 16 5	15 5	.98	.0632	.41%
15 to 20 ..	17.	1 09	.0641	.38%

fig. 52), and yet, in certain instances, presents a tendency toward diffusion (pl. 46, fig. 37). Linin fibers connect the karyosome and nuclear membrane, and these can usually be easily detected, even in the eight-nucleate cysts. The nucleus, seldom, if ever, presents the transparent appearance of the nuclei so characteristic of *C. muris*.

The majority of the cysts are spherical, though ellipsoidal (pl. 46, fig. 35), and irregularly-shaped forms (pl. 47, fig. 49) are found. The irregular shape of the cyst in figure 49, simulates the shape of a motile amoebae. One wonders if Schaudinn (1903) did not confuse irregularly-shaped cysts of this type with motile amoebae when he elaborated his theory of "schizogony" for *E. coli*.

As in *C. muris*, three races of amoebae have been determined in *C. decumani*, the smallest averaging 14μ, the next 15.5μ and the largest 17μ (see table 6). The eight-nucleate cyst is regarded as the typical mature cyst, but instances of sixteen-nucleate cysts have been found (pl. 48, fig. 52). In this case the cyst is abnormally large, being 22μ in diameter.

Budding

Instances of budding have been found in *C. decumani* in material from the mouse, from the rat, and in induced cross-infections from each of these animals to the other.

One important feature, however, differentiates the budding process in *C. decumani* from that in *C. muris*. This is the absence of its association with an excessive amount of chromatoidal material or with the general presence of chromophile ridges. A comparison of the figures at the top of plate 48 with those on plate 45 brings out a most striking difference. This feature affords important evidence in favor of the theory that a pore is normally present in the cyst wall and that at the proper juncture in the life history, this opens and permits the contents of the cyst to pass to the outside. Figure 54, plate 48, shows a first bud in the early stages of formation. Figure 55 shows a nucleus at the pore or point of emergence of the bud. It is somewhat pointed and has begun to elongate. Three other nuclei remain in the cyst and there are empty patches in the cytoplasm, the material from which has gone to form buds that have previously been discharged.

Mitosis

The general process of mitosis is similar to that of *C. muris*, but certain important differences should be noted.

Prophase.—The first indication of this phase is the enlargement of the halo or gray area around the karyosome (pl. 46, figs. 33, 34) in which chromatin granules, much smaller than the chromosomes, may appear. About this time the large, massed karyosome begins to divide into two parts (fig. 34) which move away from each other. As the portions separate, they are connected by the intradesmose (fig. 38). These two masses take up their position at either end of the somewhat elongated nucleus (pl. 47, fig. 39) and form the centrosomes. As the amount of chromatin material encrusted on the nuclear membrane becomes less during these processes of development, it is probable that a portion of this material functions in the formation and organization of the spindle and chromosomes. In the gray-staining area between the centrosomes, the spindle emerges with the chromosomes arranged on its fibers. As in *C. muris*, no linin fibers are seen in nuclei in the late prophase.

Metaphase.—Figures of the metaphase (pl. 47, figs. 41, 45) in this species exhibit four chromosomes. They are spheroidal in shape, one

being large, two medium in size, and the fourth so small that it does not stain as darkly as the others.

The processes, resulting in the formation of the anaphase and telophase, are carried out in a manner similar to those already described for *C. muris.*

ENDAMOEBA RATTI sp. nov.

This species of amoeba has been encountered in only 3 of the 288 rats examined to date and in none of the mice. As its characteristics warrant its classification with the genus *Endamoeba*, this species has been named *Endamoeba ratti* sp. nov. Permanent slides, on which are to be found stained specimens of both the cysts and free amoebae, have been available from two of these three rats.

The Motile Amoeba

Early in this investigation, a motile amoeba was observed in a normal saline preparation which presented characteristics entirely different from those exhibited by the other amoebae found in rats and mice. In comparison with amoebae of the genus *Councilmania*, the general appearance of this amoeba, when rounded, is more opaque; the structure of the endoplasm is more vacuolated and granular; and the pseudopodia never present the characteristic hyaline appearance of the pseudopodia formed by amoeba belonging to the genus *Councilmania.*

In progressive movement, the pseudopodia formed are always granular and the endoplasm constantly extends to the outermost margin of the pseudopodium (fig. C). The amoeba does not progress by a looping method, as described for *Councilmania*, but rather flows forward by a streaming movement, rounding up less often than *Councilmania.* The nucleus is often located in the extreme anterior end of the amoeba during this forward movement. The posterior, conical projection, already described in *Councilmania*, is more prominent and more constant in this species. The pseudopodia are characteristically blunt and granular.

When attached, the amoeba occasionally produces a narrow ectoplasmic margin when the pseudopodium first begins to form. This lasts only for an instant and the endoplasm immediately flows into it before its completion. A completely formed hyaline pseudopodium has never been seen, and the conical pseudopodia, common in *Councilmania*, are not formed. On the whole, the amoeboid movement of this

species of amoeba simulates very closely the movement of *Endamoeba coli* as described by Dobell (1921), and Kofoid, Swezy, and Kessel (1923*b*).

Endamoeba ratti, although possessing many of the characteristics of *E. coli*, is smaller in size, the average diameter of rounded, unencysted forms being about 19μ, while that of *E. coli* ranges from 20μ to 30μ (Dobell and O'Connor, 1921).

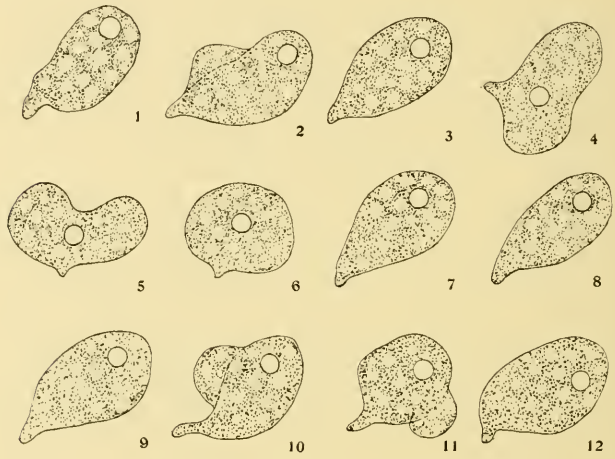

Fig. C

Free-hand sketch of *Endamoeba ratti* while in progressive movement.

The nuclear membrane is definite and is encrusted with chromatin which may present a beaded appearance or a darkly staining ring (pl. 48, fig. 57). The karyosome is excentric and may be surrounded by a clear or a gray halo (fig. 57).

The food appears to be the same as that ingested by the other non-pathogenic amoebae of the intestinal tract of rats and mice.

Encystment

As permanent slides have been procured from only two cases of infection of *Endamoeba ratti*, it has been impossible to secure a complete series of the developmental stages of the cysts. A sufficient number of the eight-nucleate cysts have been found to give a typical picture of the ripe cysts, and a few of the intervening stages have been seen.

The nuclei in the resting stage simulate very closely the typical nuclei of *E. coli* (pl. 48, figs. 58–62). The nuclear membrane is distinct and may or may not be encrusted with chromatin granules. The karyosome is in the form of a small but distinct excentric sphere. The diffuse, excentric karyosome and the heavy, peripheral chromatin blobs found in *C. decumani* have not been seen in this species. The binucleate cysts (pl. 48, fig. 59) have a large glycogen vacuole similar to the type found in *E. coli*, but the chromatoidal bodies (fig. 60) are more massed than in the type usually found in *E. coli*.

No evidence of budding has been found in the cysts thus far encountered.

The average diameter of the cysts in this species is 14.6μ. This is much smaller than the average diameter of the cysts of *E. coli* which is 17.3μ.

The conclusions to be drawn at the present time from the study of this species is that it differs from *E. coli* with respect to size, and in the massing of the chromatoidal bodies.

Endamoeba coli that have passed through rats during this investigation maintain the structural and size differences mentioned above and it is on this basis that *E. ratti* is differentiated from *E. coli*.

EXPERIMENTS IN CROSS-INFECTION

While careful study of the stained material at hand indicates decisively that *Councilmania muris* and *C. decumani* are distinct species, in the light of the successful infection of rats with the amoebae of the human intestinal tract (Kessel, 1923*b*) in which transfer the amoebae underwent no visible morphological change during the period of the experiment, cross-infection of *C. muris* and *C. decumani* was attempted from rats to rats, from rats to mice, from mice to mice, and from mice to rats. The results are tabulated in the accompanying tables.

In this experimental work the amoeba-free rats and mice were chosen by the epsom salt method as in the other infection experiments carried on in this investigation. As very young amoeba-free mice were not always on hand, it was necessary in most cases to use old mice. It has been found throughout this investigation that old rats and mice are less susceptible to infection than young stock. This suggests that the older animals are able to establish an active immunity against infection. In the series of experiments in attempting to transfer *C.*

TABLE OF DIFFERENCES BETWEEN E. COLI, C. LAFLEURI, C. MURIS, C. DECUMANI AND E. RATTI

Motile amoeba	E. coli	C. lafleuri	C. muris	C. decumani	E. ratti
Pseudopodia	Granular-vacuolated	Hyaline	Hyaline	Hyaline	Vacuolated
Size, average	20 micra to 40 micra	63 micra x 35 micra to 20 micra x 35 micra	19 micra	19 micra	19 micra
Nuclear wall and structure	Thick wall, medium amount of chromatin material	Thin wall, small amount of chromatin material	Very thin, little or no chromatin on membrane	Distinct, much encrusted, chromatin material	Distinct, medium thickness, medium amounts of chromatin
Karyosome	Small, excentric massed	Large, excentric, massed or dispersed	Dispersed, central or excentric	Sphere or crescent, massed or slightly dispersed, excentric	Massed, excentric
Encysted stages			Average of races 15.8 micra	Average of races 15.5 micra. Cysts range from 12 micra to 22 micra	Average diameter 14.5 micra
Size of cyst	16 micra to 30 micra	Average 16 micra to 20 micra			
Shape of cyst	Mostly spherical	Spherical, spheroidal, oval and irregular	Spherical or spheroidal	Spherical, spheroidal or irregular	Spherical
No. of nuclei in ripe cyst	8–16	8–16	8	8–16	8
Karyosome	Small, excentric massed	Large, generally central, or but slightly excentric, dispersed	Large, central, dispersed	Large, excentric, slightly dispersed	Small, excentric, massed
Chromosomes	Six	Eight	Six	Four	Not known
Chromatoidal body	Splinter-like or acicular	Filamentous or fasciculate. Massed in later stages	Bundles of splinters with jagged ends	Bundles with rounded ends or small spheres	Bundles with splintered or rounded ends
Budding	Unknown	From pore in chromophile ridge	From pore in region of chromophile ridge	From pore in cyst wall	Unknown

decumani from mouse to mouse and from mouse to rat, faeces were fed from the same mouse to both the mice and the rats. As the result was positive in rats and negative in the mice, the indication is that the reason for the negative results in the mice is to be traced to some condition within the mice rather than to a non-viable condition of the cysts. In transferring *C. muris* from mouse to mouse and from mouse to rat, faeces from the same mouse were used to feed both the rats and mice. As similar results were obtained in this experiment as in the previous one with reference to non-infection of the mice, similar conclusions are applicable.

TABLE 7

TABLE SHOWING RESULTS OF EXPERIMENTS IN CROSS-INFECTION OF *C. muris* AND *C. decumani* FROM RATS TO MICE AND FROM MICE TO RATS

C. muris

	Period of feeding	Number of animals	Positive	Negative	Amoeba found	Conclusion
Mouse to mouse	3 weeks	2	0	2	0	Immunity
Mouse to rat ..	4 weeks	4	4	0	C. decumani	No change
Rat to rat	3 months	5	3	2	C. decumani	No change
	3 weeks	3	2	1	C. decumani	No change
Rat to mouse .	2 months	2	0	2	0	Immunity
	3 weeks	2	0	2	0	Immunity

C. ratti

	Period of feeding	Number of animals	Positive	Negative	Amoeba found	Conclusion
Mouse to mouse	3 weeks	2	0	2	0	Immunity
Mouse to rat	3 weeks	3	2	1	C. muris	No change
Rat to rat	1 feeding	3	3	0	C. muris	No change
Rat to mouse...	2 months	4	2	2	C. muris	No change
	2 months	2	0	2	0	Immunity
	1 feeding	2	2	0	C. decumani and C. muris	Mixed*

* This case of mixed infection is thought to be due to a positive infection of *C. decumani* which was overlooked in the examination with epsom salt.

The facts that *C. decumani* has been successfully transferred from rats to rats and from mice to rats without any perceptible morphological change, and that *C. muris* has also been transferred from rats to rats, from mice to rats, and from rats to mice without a morphological change, are further evidence in favor of the constancy of the characters upon which both of these species are established.

It is much more difficult to force mice to take epsom salt than it is to induce rats to take it. Because of this fact, one is not always certain that the experimental animal is amoeba-free, as is the case with the experimental rats. Further, the salt method of obtaining amoeba-free animals has not been conclusively tested with mice, as it has with rats. Attempts to infect mice with the amoebae of the human digestive tract have not met with the success that attended similar infection experiments with rats. It is thus easily understood that mice are much more difficult to work with than rats. The mixed infection of the third attempt to transfer *C. muris* from rat to mouse may be therefore attributable to a probable previous infection of *C. decumani* that had been overlooked in determining the amoeba-free mice.

Culture.—No success has attended our attempts to culture parasitic amoebae in artificial media. Although instances have been recorded in the earlier works in which *E. coli* and *E. dysenteriae* are supposed to have been cultured, it is now generally recognized that the workers were dealing in their culture amoebae with non-parasitic species (Dobell, 1919). In this investigation it was impossible to engage in extensive attempts to culture amoebae from the rat, but several brief endeavors were made.

The first medium used was that recommended by Musgrave and Clegg (1904), for *E. coli.* Attempts were made with an alkaline medium in an anaerobic environment only and were unsuccessful. Wenyon (1907) had previously tried this medium both aerobically and anaerobically, but without success.

In order to simulate more closely the environmental conditions of the caecum in which the amoebae live in greatest numbers, at the suggestion of Dr. T. D. Beckwith of the Department of Bacteriology of this University, the hydrogen ion concentration of the caeca of several rats was taken. The caeca of eight rats non-infected with amoebae, and of eight rats that showed amoebic infection, were tested. The average pH of the caecal contents of both amoebae-free rats and of rats with positive amoebic infection was 6.35.

Rettger and Cheplin (1921) showed that the pH of the faecal material from the caecum and colon ranged from 5.5 to 6.7 in the various rats they tested. They failed to differentiate between the pH of the caecum and other parts of the digestive tract. Furthermore, their work was carried on in connection with the intestinal flora of rats and no record was given of the presence or absence of amoebae.

At the suggestion of Dr. I. C. Hall of the Department of Bacteriology of this University, one series of rats of known amoebic infection with *C. muris* or *C. decumani* was fed on a meat diet, only, for two weeks, and another series of rats known to be infected with amoebae was fed only skimmed milk for two weeks. Infections were determined by the epsom salt method (Kessel, 1923*a*). After the feeding had been continued for two weeks, the experimental animals were autopsied and examined for amoebae. Of the 15 rats fed on milk diet only, the whole number had been cleared of amoebic infection. Of the 6 rats fed on meat diet for the two weeks, 4 had been cleared of amoebic infection. Nineteen amoebae-positive rats were fed on the regular diet of table scraps and were used as a control. Of the previously infected control rats 7 were found to have been cleared of amoebic infection on terminal examination, indicating that the purgative may, at times, clear some of the rats of infection, or that an active immunity may arise.

As all the rats fed on milk diet, while only two-thirds of the rats fed on a meat diet, had been cleared of infection, it was considered that a culture medium of high animal protein content was more suitable for the amoebae than one of higher carbohydrate content such as milk. Cannon and McNease (1923) have shown that when meat is fed to white rats the reaction of the contents of the caecum is practically neutral. Consequently, beef broth and brain media were prepared and titrated to a pH of 6.35. Motile amoebae from the caecum of newly autopsied animals and cysts were planted in these media, both in the broth and in a 0.5 per cent agar. These were incubated at 37° C. under both aerobic and anaerobic conditions. No success attended these attempts, apparently because of the putrefaction produced by the rapid growth of bacteria.

One important result derived from this phase of the work should not be overlooked and that is the fact that all the rats fed on a milk diet, only, for two weeks were amoeba-negative at the end of that time, while of the control rats only 27 per cent were negative. It is interesting to note that Hegner (1923) obtained reductions in the number of flagellates in rats fed on a milk diet though his rats were not entirely rid of the infection.

It has been shown by Rettger and Cheplin (1921) and Cannon and McNease (1923) and others preceding them that feeding milk only to rats produces a change in the flora of the digestive tract from a majority of gram-negative rods, presumably *Bacillus coli*, to a pre-

dominance of gram-positive rods, *Bacillus acidophilus.* Cannon and McNease have further shown that the pH of the caeca of rats, fed on meat only, averages 7.1, while the caecal contents assume an acidity of pH 4.5 when the animals are fed on milk only.

It would seem that one or both of two main factors are responsible for the disappearance of the amoebae from the digestive tract of the rats. First, it has previously been noted in this paper that amoebae ingest gram-negative bacilli rather than gram-positive bacilli. The change in the flora from gram-negative to gram-positive rods may result in the starvation of the amoebae. Second, the great increase in the acidity of the caecal contents as the result of the lactic acid produced from the milk may have an antagonistic effect on the amoebae.

DISCUSSION

Classification and systematic relationships.—Differentiation into species of animals so minute as amoebae and with such a limited number of characteristics has always been a difficult problem. In non-parasitic amoebae, species characters are generally based on the type of pseudopodia and the nuclear structure (Schaeffer, 1920). Schaeffer said that these characteristics are hereditary and are due to "fundamental chemical structure of the protoplasm which is specific for the species." In speaking of parasitic amoebae, Dobell (1921) states that the important structural characters distinguishing the various genera and species are supplied by the nuclear apparatus and by the cysts. Differences in the types of pseudopodia formed and structural differences in the protoplasm are also generally regarded as bases for differentiation of species. It is recognized by the writer that much unwarranted differentiation into species has occurred in the past, involution forms of the same species having been regarded as showing specific differences. The mechanism of mitosis is similar in all species of parasitic amoeba and in certain phases of mitosis it is often impossible to distinguish different species from each other. The tendency to confuse these different phases should be carefully guarded against and only those characteristics which are permanent at some definite phase of the life cycle should be considered in differentiation of species; e.g., the resting stage of the nucleus.

The further facts that it is possible for the same species of parasitic amoeba to be transferred from host to host without any recognizable morphological change (Kessel, 1923*b*) and that transfer of

Councilmania muris and *C. decumani* recorded in this investigation has been successful indicate that the environment in which a parasitic amoeba may be found is not necessarily indicative of its species. This possibility should always be taken into consideration before new species are named and described.

In this investigation, the different species of amoebae found in rats and mice have been separated mainly on differences in pseudopodial formation and protoplasmic structure, on differences in the nuclei found in the resting stage, for in the cysts a condition exists primarily in which environmental factors are least influential, on the differences in chromosome count, and on differences in the structure of the chromatoidal bodies.

Councilmania muris belongs to the genus *Councilmania* because it possesses chromophile ridges, reproduces by budding from the cyst in the caecum, produces hyaline pseudopodia, and possesses a dispersed karyosome. It differs from *C. lafleuri* in size, and in the possession of a very thin nuclear membrane upon which little or no chromatin is ever encrusted. The granules in the karyosome are smaller and are more dispersed in motile amoebae of this species than in those of *C. lafleuri*. It has six chromosomes, while *C. lafleuri* has eight.

Councilmania decumani belongs to the genus *Councilmania* because of its reproduction by budding from the cyst, because it produces hyaline pseudopodia, and because of its dispersed karyosome. It differs from *C. lafleuri* and from *C. muris* in that the karyosome is excentric and is more massed than in either of these species, and in that the nuclear membrane is more distinct and is encrusted with large chromatin blobs. There is less chromatoidal substance in this species than in either *C. lafleuri* or *C. muris*, and there is not so pronounced a chromophile ridge. This species has four chromosomes.

Up to the present time no amoeba possessing granular and vacuolated pseudopodia has been described from the rat or mouse. Balfour (1922), however, did mention that on two occasions during his investigation cysts of the *E. coli* type were noted from black rats. As he also suggested that they were possibly the same as those found by Brug (1919), we conclude that Balfour had no opportunity to differentiate between *E. coli* of man and the *coli*-like and other amoebae of the rat.

If differences in the structure of pseudopodia have specific values, as the extensive work of Penard (1904) and Schaeffer (1921) indicates, it then follows that an amoeba which possesses hyaline pseudo-

podia cannot belong to the same species as an amoeba that forms granular pseudopodia. This difference alone should be sufficient evidence for the establishment of a new species.

During this investigation, an amoeba has been encountered in the intestinal tract of the rat which moves by the formation of granular and coarsely vacuolated pseudopodia. Except in very rare instances, when the amoeba is very sluggish in movement and for the most part rounded, when a narrow hyaline margin of ectoplasm may occur for an instant only, this amoeba shows absolutely no differentiation between ectoplasm and endoplasm. It has never been observed to form a pseudopodium, completely hyaline, which is the only type of pseudopodium ever observed in *Councilmania*. The type of pseudopodial formation observed in this amoeba resembles that described by Dobell (1921) for *E. coli*.

The nuclear structure of this amoeba also differs from the nuclear structure of *Councilmania* in that the karyosome is solid rather than diffuse. It further differs from species of amoebae found in the rat that belong to the genus *Councilmania* in that reproduction by budding from the cyst in the caecum has not been observed in it. Because of these differences, it is concluded that this species of amoeba cannot be placed in the genus *Councilmania*.

Since the pseudopodia are of the granular type and since the karyosome is massed and excentric, as in the type species *E. blattae* (Bütschli) and also in *E. coli* (Lösch), we conclude that this amoeba from the rat should be placed in the genus *Endamoeba*.

This amoeba found in the rat differs from *E. coli* of man in size and in the type of chromatoidal body, which simulates the chromatoidal body of *C. decumani* more than the chromatoidal body of *E. coli*. There is also less tendency for the chromatin material encrusted on the nuclear membrane to arrange itself in a bead-like structure. It is usually encrusted in a solid ring, or in granules spaced widely apart. This, however, may be physiological rather than specific. Because of these differences between the amoeba possessing granular pseudopodia that is found in the rat and *E. coli* of man, it is proposed to call this amoeba *Endamoeba ratti* sp. nov.

Pseudopodial formation.—The structure of the protoplasm and the type of pseudopodia formed by the various amoebae, both free-living and parasitic, must be associated with the biochemical construction of the protoplasm of which the specific amoebae are composed. It has been suggested by some investigators that the difference in pseudo-

podial formation may be merely a difference of the moment, dependent upon the immediate environmental condition. It is, however, more generally accepted that the structure of the protoplasm and the type of pseudopodia formed are constant in the normal environment and may be used as characters for differentiating species.

In the amoebae previously described as parasitic in rats and mice, it is significant to note that, without exception, they have been described as possessing hyaline pseudopodia. It is mentioned by every investigator that there is a sharp line of demarcation between the endoplasm and ectoplasm in the formation of pseudopodia. It seems that the work has been carried on over a sufficient period of time and by men of sufficiently sound methods in this line of investigation to conclude that if there had been any possibility of change from a hyaline to a granular type of pseudopodium in this species of amoeba, it would, at some time, have been noticed. It therefore seems from the work of the past and from the present investigation that the characteristic of hyaline pseudopodial formation is a definite and constant general character in these two amoebae, *C. muris* and *C. decumani*.

As already quoted from Dobell (1921), the growing impression is that in *Endamoeba coli* "no sharp line of demarcation separates the ectoplasm from the endoplasm." With the exception of Rudovsky, all the previous investigators mention size as the chief differentiating character between *E. coli* and the amoebae of the rat and mouse that have been described by them. It follows that, if pseudopodial formation is to be regarded as a specific character, *E. coli* and the amoebae of the rat and mouse described as *Councilmania muris* and *C. decumani* cannot be identical.

The possibility that one species of amoeba may form hyaline pseudopodia at one time and granular pseudopodia at another time has been constantly borne in mind during the present investigation, but no evidence supporting this theory has been found in any of the cases examined. One instance, which the writer considers to be a case of a mixed infection, was found in which amoebae possessing hyaline pseudopodia and other amoebae possessing granular pseudopodia were found in the same field of the microscope. The actively motile forms of the amoebae exhibiting hyaline pseudopodia in this particular instance rounded up after being under observation about ten minutes, and showed no further activity, while the amoebae with granular pseudopodia remained active for an extended length of time. This tendency of amoebae to become inactive after being in the unusual environment of the normal saline smear for a short period is char-

acteristic of the species belonging to the genus *Councilmania* found in the rat. On the other hand, *Endamoeba coli* is not so easily affected by the change of environment and may continue its activity in normal saline on the warm stage for several hours (Kofoid, Swezy, and Kessel, 1923*b*).

Evolutionary source of infection of Councilmania of man.—The source of the infection of the parasites of man is an important problem. While it is impossible to state in all instances the source from which man originally acquired his parasitic infections, the evolutionary origin of certain protozoan infections is at least suspected. *Iodamoeba bütschlii* has been described as occurring in the pig and it is suspected (Kofoid, 1923) that man acquired his original infection from that source. The wild game in Africa is thought to be the source of infection to man of the causative organisms of African sleeping sickness and Reichenow (1920) has recently claimed that monkeys are a dangerous source of infection to man, of the organisms causing malaria.

Rats and mice have long since been regarded as among the most annoying pests of mankind. On account of their tendency to inhabit the dwelling places of the human race, they have from earliest times doubtless been brought into very close proximity with the substances used by man for food. It seems very likely that these rodents may also have functioned in the evolutionary origin of man's parasitic *Councilmania* in the past (Kofoid, 1923).

The remarkable similarity between the species of amoebae belonging to the genus *Councilmania* found in man and the two species of that genus found in rats and mice leads one to suspect that these animals may be the original source of infection by the genus *Councilmania* in man; i.e., man may have originally acquired his infection of this species of amoeba from rodent hosts. This suspicion leads one to the consideration of the question of the obligate specificity between host and parasite which has been discussed by Kessel (1923*b*). The successful experimental transfer of *C. muris* and *C. decumani* recorded in this paper affords valuable evidence against the specificity of parasitic amoebae to a given host.

Immunity.—The question of the establishment of an active immunity to amoebiasis is a new one. No experimental work with respect to this question has been done with the amoebae of man. Yet, in the examination of rats during this investigation, the question of the possibility of certain animals being immune to infection has presented itself on several occasions.

First, the incidence of infection among old rats is much lower than among young rats or rats of middle age. Secondly, in 27 per cent of the cases in this investigation rats and mice which had a previously determined amoebic infection have been known to clear themselves of the infection without the administration of curative measures. Thirdly, among rats and mice subjected to precisely the same feeding experiments, some animals became infected while others remained free from amoebic infection. No data were kept for the rats, but among the mice those that did not become infected as a result of the feeding experiments were old mice that had been diagnosed as amoeba-free at the beginning of the experiment.

These results have been obtained from experiments bearing on subjects other than immunity, but they present striking suggestions and open the way for further investigation along this line.

Reproduction.—In so far as is known, all reproduction in amoebae is by the asexual method. There has been no recent evidence to support the earlier idea of autogamy and no sexual cycle or syngamy has been determined. It is generally accepted that asexual reproduction may occur either in the motile amoeba or in the encysted stages.

Although Schaudinn (1903) and Mathis and Mercier (1917) described the process of multiple schizogony as occurring in *Endamoeba coli*, conclusive evidence has not been produced for this method of reproduction. It is the opinion of the writer that irregularly shaped cysts (pl. 47, fig. 49), which may simulate motile amoebae in shape, may have been interpreted as motile amoebae and the theory of schizogony based on this evidence.

Binary fission seems to be the usual method of reproduction in motile amoebae; at any rate, motile amoebae with more than two nuclei have never been seen in this investigation. Motile amoebae with two nuclei have been observed and, while they are not common, the fact that they have been found indicates that the process of binary fission is quite normal.

Probably the more usual method of reproduction of parasitic amoebae is by multiple nuclear formation within the cyst. A mononucleate amoeba encysts and by nuclear divisions produces a cyst with successively two, four, eight, or sixteen nuclei. In the three amoebae here described from the rat and mouse, eight nuclei is the common number for the mature cyst.

The question of periodicity of encystment has been discussed in an earlier paper (Kessel, 1923*a*), but the growing impression among investigators is that there is no regular periodicity of similar intervals

in the formation of cysts and that no regular periodical life cycle is apparent for parasitic amoebae. Kofoid (1923) has suggested that encystment occurs as the result of the presence of reserve food rather than for the purpose of protection to the protozoan. Protection is afforded while passing from one animal to another, but this is a secondary function following from the formation of the cyst. As cysts are found in the caecum as well as in the faeces, there are indications that reproduction by multiple fission within the cyst in *Councilmania* may occur within the host independent of discharge. The fact that budding from the cyst apparently in the caecum of the host in which the cysts were formed has been found in *Councilmania muris* is further evidence in support of this theory.

Mitosis and the mechanism of heredity.—Early workers in protozoology expressed the opinion that cell division among the Protozoa was affected by amitosis. Perfection of cytological methods and further detailed work have resulted in the description of cell division by the process of mitosis for one after another of the Protozoa that had been previously thought to divide amitotically. It is very probable that mitotic cell division is the method of cell division in all normal cells in the animal kingdom and that amitotic division must be reserved merely to explan pathogenic or abnormal cell division.

If the mechanism of heredity is bound up in the complicated structures recognizable in mitotic division, one would expect to find a hereditary mechanism in the Protozoa similar to the one known in the Metazoa. Among the amoebae parasitic in man, accounts of mitosis have been given for *Endamoeba dysenteriae* by Dobell (1919), though he did not record the complete mitotic cycle, by Kofoid and Swezy (1921) for *Councilmania lafleuri*, and by Swezy (1922) for *Endamoeba coli*.

These accounts show that a characteristic spindle is formed in the amoebae with a centrosome at either pole, and that the centrosomes are connected by an intradesmose (Kofoid and Swezy, 1921). The centrosomes are formed from chromatin material within the karyosome. The nuclear membrane remains intact throughout the whole process of division and merely lengthens and constricts in the middle, finally dividing into two parts at the close of the telophase.

In the present investigation the process of mitosis has been recorded for *Councilmania muris* and *C. decumani*. It is essentially the same in these two species and similar to that in *Endamoeba coli* and *C. lafleuri*, the chief difference being that the chromosomes are smaller

in size. Exact chromosome counts, however, have been made and these are important in differentiation of species. The chromosomes found in the parasitic amoebae are relatively small and simple when compared with the chromosomes of the Metazoa. They are for the most part small spheres or ellipsoids. In this investigation we have found evidence of the precocious splitting of the chromosomes as described by Swezy (1922) for *Endamoeba coli*. The chromosomes do not retain their constancy of form during the resting stage of the nucleus, but are reformed at each mitotic division from the chromatin material scattered within the nucleus and encrusted upon the nuclear membrane. It is probably because of insufficient observation of mitotic stages of division that Wenyon (1922) seems to hold to the opinion that "no definite chromosomes are formed during the division of the nucleus" of parasitic amoebae.

The formation of an intradesmose (Kofoid and Swezy, 1921) is characteristic of the type of nuclear division in amoebae. This is formed within the nuclear membrane and thus differs from the paradesmose of the Mastigophora and from the centrodesmose of the metazoan cell.

SUMMARY

1. The present investigation was begun in order to determine whether or not rats may become infected with *Endamoeba dysenteriae*. It was found necessary first to determine the amoebae that normally inhabit the intestinal tract of rats and mice and this paper describes the three species of amoebae found in the intestinal tract of culture rats and mice. These three amoebae are:

(*a*) *Councilmania muris*, transferred from the genus *Amoeba* to which it was assigned by Grassi (1881) and by Wenyon (1907).

(*b*) *Councilmania decumani*, transferred from the genus *Endamoeba* to which it was assigned by Rudovsky (1921).

(*c*) *Endamoeba ratti* sp. nov., found in only three of the rats during the present investigation.

2. Specific differences of these amoebae are based on differences in the structure of the protoplasm and pseudopodial formation, morphological differences in nuclear structure, in chromatoidal bodies, in the formation of chromophile ridges, in the budding of cysts in the bowel by which young amoebulae are discharged from the cyst, and on the fact that the amoebae have been transferred by experiment from one rodent to another without apparent racial or morphological change.

3. *Councilmania muris* is characterized by the formation of hyaline pseudopodia; by a central, diffuse karyosome; by the occurrence of numerous and massed chromatoidal bodies having irregular ends in the mature cysts, by the frequency of chromophile ridges, by budding cysts in stools, and by the presence of six chromosomes.

4. *Councilmania decumani* is characterized by the formation of hyaline pseudopodia; by a fairly distinct nuclear membrane, heavily encrusted with chromatin material; by an excentric, diffuse karyosome, somewhat more massed than the karyosome of *C. muris;* by the presence of relatively few chromatoidal bodies which have a tendency to form rounded ends; by the relative rarity of well defined chromophile ridges; and by the process of budding of cysts in the stools.

5. *Endamoeba ratti* is characterized by the possession of granular and vacuolated pseudopodia; by a distinct nuclear membrane upon which the chromatin has a tendency to be encrusted in a regular ring or small beads; by chromatoidal bodies which resemble those of *C. muris;* and apparently by the absence of the budding process.

6. Distinct size races of *Councilmania muris* and *Councilmania decumani* have been observed, those of *C. muris* being 14.5μ, 15.7μ, and 17.7μ, respectively, and those of *C. decumani* being 14μ, 15.5μ, and 17μ, respectively.

7. The process of mitosis has been worked out in detail for the species *C. muris* and *C. decumani.* It resembles mitosis as already described for *C. lafleuri* (Kofoid and Swezy, 1921) and for *Endamoeba coli* as described by Swezy (1922). *C. muris* possesses six chromosomes while *C. decumani* has only four. These chromosomes present recognizable and constant differences in form.

8. Reproduction of parasitic amoebae may occur either by binary fission of the motile amoebae, or by multiple fission following encystment, which is the most common method. Young amoebae are extruded from the cysts of *C. muris* and *C. decumani* by a process of budding through a pore in the cyst wall in the caecum and stools by a process similar to that described by Kofoid and Swezy (1921) for *Councilmania lafleuri.* No evidence of buds has been found to date for *Endamoeba ratti.*

9. The possibility of the establishment of immunity to amoebic infection by the rodent host is suggested by the facts that the incidence of amoebic infection is higher in younger animals than in old ones, that rats and mice may clear themselves of amoebic infection without apparent treatment, and that it has been difficult to establish an

experimental infection in old animals that have previously been determined to be amoeba-free.

10. The fact that the amoebae common in the digestive tract of rats and mice may be transferred by experimental infection from one species of rodent to another without immediate morphological or racial change, and also that the common amoebae of the human digestive tract may be transferred to rodents without noticeable morphological change affords valuable evidence:

(*a*) In favor of the morphological constancy of species of parasitic forms.

(*b*) Against the theory of host specificity of parasites.

11. The fact that all rats of known positive amoebic infection that have been fed on a milk diet for two weeks have cleared themselves of infection while only 27 per cent of the control rats fed on table scraps have been cleared of infection indicates that a diet of milk only is for some reason antagonistic to the normal amoebic infection of the rat.

Permanent slides, representing paratypes of species *Councilmania muris*, *Councilmania decumani*, and *Endamoeba ratti* are deposited in the laboratory of the Department of Zoology, University of California.

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LITERATURE CITED

Balfour, A.
1922. Observations on wild rats in England, with an account of their ecto- and endoparasites. Parasit., **14**, 282–298, pls. 23–24, 2 figs. in text.

Brug, S. L.
1919. De entamoeben van de rat. Geneesk. Tijd. Nederlandsch-Indië, 59, 1–15, pl. 1–2.

Bütschli, O.
1878. Beiträge zur Kenntniss der Flagellaten und einiger verwandten Organismen. Ztschr. wiss. Zool., **30**, 206–281, pls. 11–15.

Cannon, P. R., and McNease, B. W.
1923. Factors controlling intestinal bacteria. The influence of hydrogen-ion concentration on bacterial types. Jour. Inf. Dis., **32**, 175–180.

Chatton, E.
1917. L'éclosion des kystes et les premiers stades de l'évolution de l'amibe dysentérique humaine chez le chat. Bull. Soc. Path. Exot., **10**, 834–841, 9 figs. in text.

Dobell, C.

1917. Reports upon investigations in the United Kingdom of dysentery cases received from the Eastern Mediterranean. 1. Amoebic dysentery and the protozoological investigation of cases and carriers. Brit. Med. Res. Com., 4 (London, Sir Joseph Caston & Sons), 85 pp.

1919. The amoebae living in man. (London, John Bale, Sons and Danielsson) vi + 155 pp., 5 pls.

Dobell, C., and O'Connor, F. W.

1921. The intestinal Protozoa of man. (*Ibid.*), v + 211 pp., pls. 1–8, 2 figs. in text.

Grassi, B.

1881. Intorno ad alcuni Protisti endoparassitici ed appartinenti alle classi dei flagellati, lobosi, sporozoi e ciliati. Atti Soc. Ital. Sci. Nat., 24, 135–224, pls. 1–4.

Grinnell, J.

1923. A systematic list of the mammals of California. Univ. Calif. Publ. Zool., 21, 313–324.

Hegner, R. W.

1923. The effects of changes in diet on the incidence, distribution and number of certain intestinal Protozoa of rats. Am. Jour. Hyg., 3, 180–200, pl. 6.

Hinton, M. A. C.

1920. Rats and mice as enemies of mankind (London, British Museum Natural History), Ex. Se., 8, ix + 67 pp., pls. 1–2, 6 figs. in text.

Huber, G. C.

1912. The development of the albino rat, *Mus norvegicus albinus*. Jour. Morph., 26, 1–142, 42 figs. in text.

Hyman, L. H.

1917. Metabolic gradients in amoeba and their relation to the mechanism of amoeboid movement. Jour. Exp. Zool., 24, 55–99, 14 figs. in text.

Kessel, J. F.

1923*a*. Methods of securing amoebae-free rats for experimental infection with intestinal amoebae. Univ. Calif. Publ. Zool., 20, 401–408.

1923*b*. The experimental infection of rats and mice with human intestinal amoebae. *Ibid.*, 20, 409–432, pls. 38, 39.

Kofoid, C. A.

1923*a*. Amoeba and man. Univ. Calif. Chron., 25, 149–174 and 291–312, pls. 1–6.

1923*b*. The life cycle of the Protozoa. Science, 57, 397–408.

Kofoid, C. A., Kornhauser, E. I., and Swezy, O.

1919. Criteria for distinguishing the *Endamoeba* of amoebiasis from other organisms. Arch. Int. Med., 24, 35–50, 43 figs. in text.

Kofoid, C. A., and Swezy, O.

1920. On the prevalence of carriers of *Endamoeba dysenteriae* among soldiers returning from overseas service. New Orleans Med. and Surg. Jour., 73, 4–11.

1921. On the free, encysted, and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine. Univ. Calif. Publ. Zool., 20, 169–198, pls. 18–22, 3 figs. in text.

Kofoid, C. A., Swezy, O., and Kessel, J. F.

1923. The genus *Councilmania*, budding amoebae parasitic in man and rodents. *Ibid.*, 20, 431–445, 10 figs. in text.

Lapage, G.
1922. Cannibalism in *Amoeba vespertilio* (Penard). Quart. Jour. Micr. Sci., **66**, 669–710, pls. 28–29, 3 figs. in text.

Lynch, K. M.
1915. The rat as a carrier of dysenteric amoeba. Jour. Am. Med. Assoc., **65**, 2232–2234.

Mathis, C., and Mercier, L.
1917. La schizogonie chez les entamibes intestinales de l'homme. Bull. Soc. Path. Exot., **10**, 311–322, 15 figs. in text.

Penard, E.
1904. Quelques nouveaux Rhizopodes d'eau douce. Arch. Prot., **3**, 391–422, 11 figs. in text.

Rettger, L. F., and Cheplin, H. A.
1921. The intestinal flora with special reference to the implantation of *Bacillus acidophilus* (New Haven, Yale University Press), pp. 135, pls. I–8.

Richenow, E.
1920. Über das Vorkommen der Malariaparasiten des Menschen bei den afrikanischen Menschenrassen. Centrbl. f. Bakt., Abt. I, **85**, 2–7–216, 1 pl., 3 figs. in text.

Rudovsky, F.
1921. Über Rattenamöbiase. Wien. Tierärzt. Monatsber., **8**, 1–9, pl. 7.

Schaeffer, Asa A.
1920. Amoeboid movement (Princeton, University Press), v + 156 pp., 46 figs. in text.

Schaudinn, F.
1903. Untersuchungen über die Fortpflanzung einiger Rhizopoden. Arb. Kais. Gesundh., **19**, 547–576.

Swezy, O.
1922. Mitosis in the encysted stages of *Endamoeba coli* (Löseh). Univ. Calif. Publ. Zool., **20**, 313–332, pls. 29–31.

Taylor, C. V.
1923. The contractile vacuole, an example of the sol-gel reversibility of protoplasm. Jour. Exp. Zool., **37**, 3 pls. 11 figs. in text.

Walker, E. L.
1908. The parasitic amoebae of the intestinal tract of man and other animals. Jour. Med. Res., **17**, 379–460, pls. 21–24.

Wenyon, C. M.
1907. Observations on the Protozoa in the intestine of mice. Arch. f. Prot., Suppl. 1, pp. 169–201, pls. 10–12.
1922. Supposed new endamoebae of man. Trop. Dis. Bull., **19**, 19–22.

Wenyon, C. M., and O'Connor, F. W.
1917. Human intestinal Protozoa in the Near East (London, Wellcome Bureau of Scientific Research), 218 pp., pls. 1–4.

Wilson, C. W.
1916. On the life-history of a soil amoeba. Univ. Calif. Publ. Zool., **16**, 241–292, pls. 18–23.

Yoshida, K.
1918. The encystment of dysentery amoebae *in vitro*. Jour. Exp. Med., **28**, 348–413, pls. 30–43.

EXPLANATION OF PLATES

All figures are made with camera lucida from smears fixed in hot Schaudinn's fluid and stained with iron-haematoxylin. × 2500.

PLATE 43

Councilmania muris

Fig. 1. Motile amoeba, showing hyaline pseudopodia. Undigested food is in some of the vacuoles. The large, dark-staining mass in the vacuole to the right of the nucleus is probably *Sphaerita*.

Fig. 2. Attached motile amoebae forming balloon-like hyaline pseudopodia.

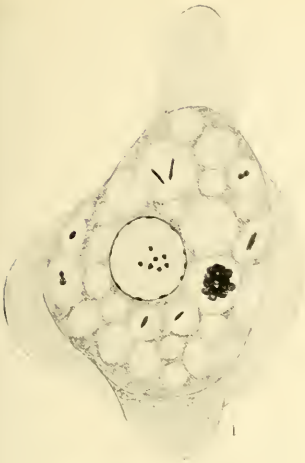
Fig. 3. Amoeba with typical hyaline pseudopodium formed as if in progressive movement. Note the posterior, root-like projection. The nucleus shows the thin membrane and diffuse karyosome, characteristic of this species.

Fig. 4. Rounded, motile amoeba, with partly digested *Chilomastix* in the large food vacuole.

Fig. 5. Rounded, pre-cystic amoeba. Note the absence of food in the vacuoles and a small number of large vacuoles, indicating that smaller ones have coalesced to form the large ones.

Fig. 6. Rounded, pre-cystic amoeba with three large vacuoles. Karyosome is composed of diffuse granules and has a gray area in center. Note the short intradesmose.

Fig. 7. Mononucleate cyst with large glycogen vacuole and nucleus in early prophase. Small chromatoidal bodies around the margin of the vacuole.



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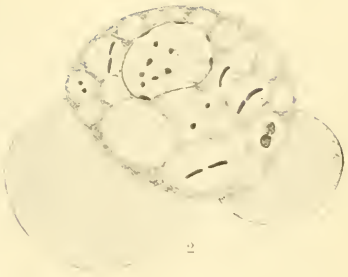

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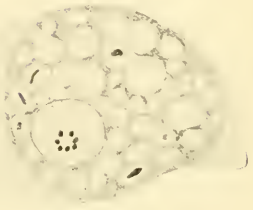

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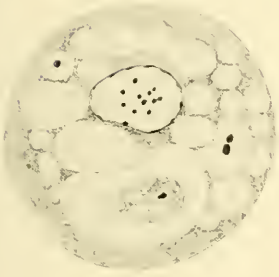

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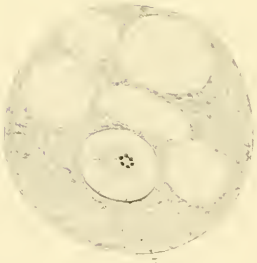

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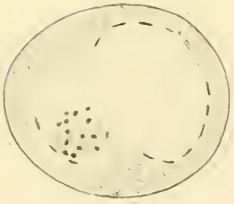

7

PLATE 44

Councilmania muris

Fig. 8. Mononucleate cyst with glycogen vacuole showing nucleus in late prophase.

Fig. 9. Mononucleate cyst without glycogen vacuole. The upper chromosomes represent a metaphase and lower ones are in early anaphase.

Fig. 10. Large mononucleate cyst with glycogen vacuole. Nucleus in telophase.

Fig. 11. Binucleate cyst showing glycogen vacuole. The upper nucleus represents a resting stage with the lower nucleus in prophase, showing intradesmose connecting centrosomes.

Fig. 12. Binucleate cyst with glycogen vacuole. Upper nucleus is in anaphase, showing six divided chromosomes and the centrosomes at either end of the spindle. The lower nucleus in very early prophase.

Fig. 13. Binucleate cyst with upper nucleus in early anaphase and lower nucleus in late anaphase.

Fig. 14. Binucleate cyst with the nuclei in anaphase showing massed chromatoidal body formed between margin of glycogen vacuole and cytoplasm.

Fig. 15. Cyst with three nuclei showing diffuse karyosome. Large nucleus is the result of the first division, the two small nuclei the result of the second division. Chromatoidal bodies are arranged between the glycogen vacuole and cytoplasm.

Fig. 16. Cyst, showing the upper nucleus in very late telophase, the lower nuclei having just separated. The diffuse condition of the karyosome is apparent in each nucleus.

Fig. 17. Four-nucleate cyst without glycogen vacuole. Three spherical nuclei are in prophase while the elongated nucleus showing chromosomes is in metaphase. Centrosomes are shown at either end of the spindle and are connected by the intradesmose.

Fig. 18. Five-nucleate cyst, without glycogen vacuole. The two larger nuclei on the left are the result of the second nuclear division, the two smaller nuclei on the right the result of the third nuclear division. The middle nucleus is in a late telophase.

Fig. 19. Eight-nucleate cyst without chromatoidal body. Nuclei show characteristic thin nuclear membrane and diffuse karyosome.

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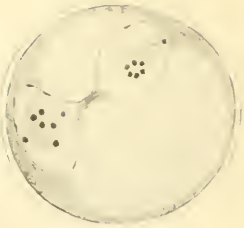

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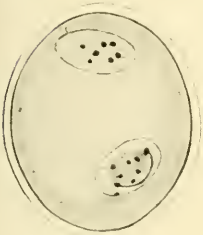

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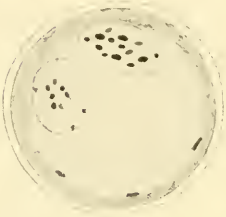

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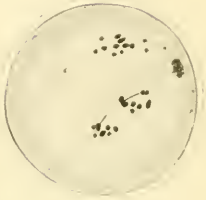

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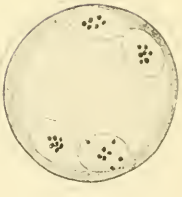

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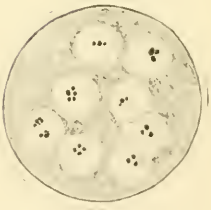


19

PLATE 45

Councilmania muris

Fig. 20. Ellipsoidal cyst with eight nuclei showing chromophile ridge extending the full length of the cyst.

Fig. 21. Spherical eight-nucleate cyst with chromophile ridges on either side and chromatoidal body in the center.

Fig. 22. Ellipsoidal cyst with bud protruding from the region of the chromophile ridge. One nucleus is located in the bud, four nuclei are left in the cyst, and a small amoebula is in close proximity to the cyst, having just previously budded off. Note the empty space in the cytoplasm and the general thin appearance of the cytoplasm, indicating that part of the cytoplasm has previously passed out of the cyst in forming the earlier amoebulae.

Fig. 23. Large, eight-nucleate cyst without chromatoidal bodies, bud, or chromophile ridge.

Fig. 24. Smaller, eight-nucleate cyst with long chromatoidal body in middle of cyst. The karyosomes are widely dispersed.

Fig. 25. Eight-nucleate cyst, showing chromophile ridge.

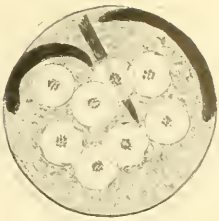
Fig. 26. Eight-nucleate cyst with bud showing chromatoidal body and typical dispersion of the karyosome toward the region of the pore, and remnants of chromophile ridges. One nucleus is in the bud.

Fig. 27. Seven-nucleate cyst showing bud, large, massed chromatoidal body, and chromophile ridges in the region of the pore.

Fig. 28. Six-nucleate cyst with one nucleus in the bud. Chromatoidal body and chromophile ridge are present.

Fig. 29. Eight-nucleate cyst with bud and showing peculiarly curved chromatoidal body in middle of cyst. Chromophile ridge is in the region of the pore.

20



21

22b

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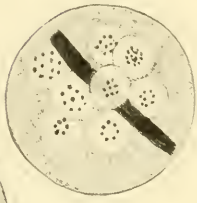

24

23

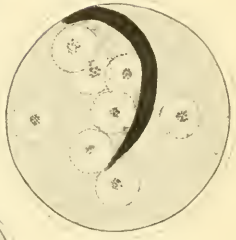

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PLATE 46

Councilmania decumani

Fig. 30. Large, rounded motile amoeba with partly digested *Chilomastix* in the upper large vacuole and a small amoeba in the lower vacuole. The nucleus shows an excentric karyosome which is surrounded by a halo.

Fig. 31. Large, rounded motile amoeba with two nuclei.

Fig. 32. Medium size, motile amoeba, showing hyaline pseudopodia extended. The nucleus shows a crescent-shaped, excentric karyosome.

Fig. 33. Large mononucleate cyst without glycogen vacuole, and small spherical chromatoidal bodies in the cytoplasm.

Fig. 34. Mononucleate cyst with glycogen vacuole. The karyosome has begun to separate and is connected by the intradesmose. Heavy peripheral chromatin is encrusted on the nuclear membrane.

Fig. 35. Ellipsoidal mononucleate cyst with glycogen vacuole. The spindle shows most of the chromosomes in early anaphase and the centrosomes are connected by the intradesmose. Note the blobs of chromatin encrusted on the nuclear membrane.

Fig. 36. Small, binucleate cyst with glycogen vacuole. The spherical nucleus is in the resting stage. The ellipsoidal nucleus is in the early prophase.

Fig. 37. Binucleate cyst showing nuclei slightly elongated in very early prophase prior to the division of the karyosome.

Fig. 38. Binucleate cyst with chromatoidal bodies near the margin of the glycogen vacuole. The centrosomes are beginning to pull apart and are connected by the intradesmose.

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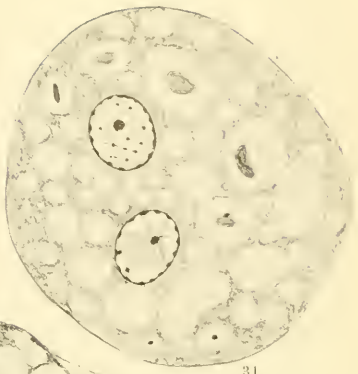

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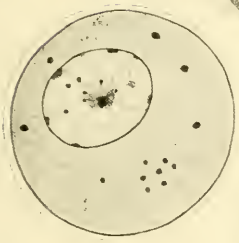

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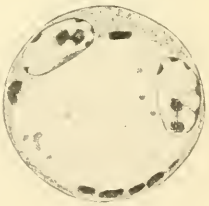

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PLATE 47

Councilmania decumani

Fig. 39. Binucleate cyst with nuclei in prophase. Centrosomes are arranged at either end of the spindle and are connected by the intradesmose. Chromatoidal bodies are arranged around margin of the glycogen vacuole.

Fig. 40. Binucleate cyst with the nuclei in prophase. Note the area in the region of the spindle in which are located four chromatin granules. The centrosomes are connected by the intradesmose.

Fig. 41. Binucleate cyst with chromosomes arranged on spindles in metaphase. Heavy peripheral chromatin is formed on the nuclear membrane.

Fig. 42. Binucleate cyst with small glycogen vacuole. Chromatoidal bodies of varying shapes are in the cytoplasm. Chromosomes on spindle are in anaphase.

Fig. 43. Cyst without glycogen vacuole and with three nuclei. The large nucleus is the result of the first division, and the small nuclei the result of the second nuclear division. Chromatoidal bodies are in the shape of small spheres and splinters.

Fig. 44. Cyst with three nuclei and large glycogen vacuole. The nuclei represent different phases of mitosis.

Fig. 45. Four-nucleate cyst without glycogen vacuole. Two lower nuclei show the chromosomes in metaphase. One large chromosome, two medium-sized chromosomes, and one small chromosome are evident.

Fig. 46. Cyst with five nuclei. The three large nuclei have not undergone second division.

Fig. 47. Irregularly shaped cyst with eight nuclei and no chromatoidal body. The nuclei show the typical resting stage with excentric karyosome and large blobs of chromatin encrusted on the nuclear membrane.

Fig. 48. Spherical, eight-nucleate cyst with typical chromatoidal bodies having irregular ends.

Fig. 49. Irregularly shaped eight-nucleate cyst with small chromophile ridge.

Fig. 50. Spherical eight-nucleate cyst containing small, spherical chromatoidal body and large massed chromatoidal bodies with rounded ends.

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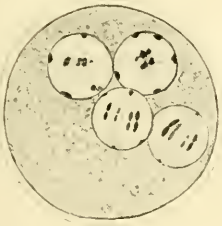
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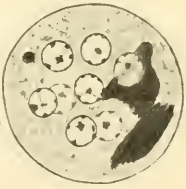
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PLATE 48

Councilmania decumani, figs. 51–56

Fig. 51. Spherical, eight-nucleate cyst showing small chromophile ridge.

Fig. 52. Very large, sixteen-nucleate cyst showing no chromatoidal bodies.

Fig. 53. Eight-nucleate cyst showing bud. One nucleus is in the bud and the second nucleus is in the region of the pore.

Fig. 54. Eight-nucleate cyst showing bud just beginning to protrude.

Fig. 55. Cyst containing four nuclei. One nucleus is in the region of pore and shows elongation prior to its entrance into the bud. The cytoplasm in the cyst is thin and shows regions from which other buds have formed.

Fig. 56. Cyst with bud showing eight nuclei.

Endamoeba ratti, figs. 57–62

Fig. 57. Motile amoeba showing vacuolated and granular pseudopodia. The karyosome is excentric and is surrounded by a halo.

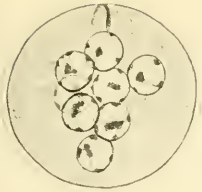
Fig. 58. Eight-nucleate cyst showing no chromatoidal body. The karyosome is massed and excentric.

Fig. 59. Binucleate cyst showing glycogen vacuole and two small, chromatoidal bodies.

Fig. 60. Eight-nucleate cyst showing large, massed chromatoidal body with splintered ends.

Fig. 61. Eight-nucleate cyst showing massed chromatoidal body, the margins of which are rounded. Small chromatin beads are arranged on the nuclear membrane.

Fig. 62. Eight-nucleate cyst showing typical massed, excentric karyosomes in the nuclei. The peripheral chromatin on nuclear membranes is heavy.



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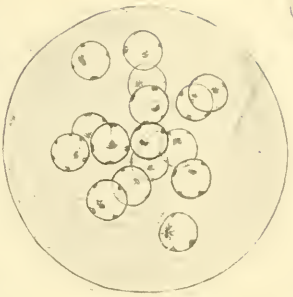

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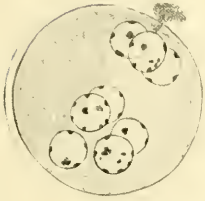

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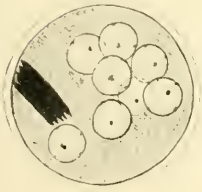

60

61

62

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INDEX

Univ. Calif. Publ. Zool., vol. 20.

Titles of papers and names of new systematic units in boldface. Acknowledgment of authors cited is made for each paper under the heading "Literature Cited."

Alveolar layer, in *Trichonympha*, 54; in *Leidyopsis*, 105, 111.
Amitosis, 460.
Amoeba coli, 490.
dysenteriae, 170.
hyalina, 323.
muris, 433, 434, 490.
Amoeba, intestinal, of man, 169–198, 313–332, 431–445; in bone marrow, 301–307; difficulty of culture, 313; sexual process in, 324; discharge of, in rats, 402; evacuation through purgative, 403; incidence of infection in rodents, 405, 406, 494, 496, 527; rate of infection and age of hosts, 407, 408, 423, 494, 527; inoculation in guinea pigs, 410, in rabbits, 410; commonly in caecum of rodents, 421; parasitic in rats and mice, 489–544; reproduction, 527–528.
Arthritis deformans, 301–307.
Ascaris toxin, 235–242.
Askaron, proteose toxin from *Ascaris*, 235.
Astasia levis, 463.
proteus, 448.
Astasiopsis distorta, 448.
Autogamy, in *Endamoeba coli*, 324.
Axostyle, in *Giardia enterica*, 208; in *Pentatrichomonas*, 380, 384; in *Trichomonas*, 373.
Balantidium, systematic position of genus and 22 known species, 248; comparative measurements, 252–254.
coli, 243–300.
entozoön, 244.
suis, 243–300.
Banks, Nathan, acknowledgment, 2.
Basal apparatus, in *Balantidium*, 271.
Beckwith, T. D., acknowledgment, 477.
Bercovitz, N., 365–371.
Bilateral symmetry, in polymastigote flagellates, origin of, 215.
Binary fission, *see* Fission, binary.
Biochemical changes, susceptibility of flagellates to, 29, 43.
Blastocystis, 324, 376.
Blepharoplast, in *Chilomastix*, 124, primary, 124, secondary, 125, and tertiary, 125; in *Giardia*, 206; in *Menoidium*, 451, 459; in *Pentatrichomonas*, 378; in *Streblomastix*, 8, 14; in *Trichomitus*, 23, 31, 32; in *Trichonympha*, 70–71.
Board of Research, University of California, acknowledgment, 375, 432.
Bodo intestinalis, 163.
Bone marrow, in Arthritis deformans, *Endamoeba dysenteriae* in, 301–307.
Boyers, L. M., 309–312; acknowledgment, 375.
Budding, in *Councilmania*, 181, 431–445, 508, 514.
Bursaria, 270.
California State Board of Health, Division of Parasitology of the Bureau of Communicable Diseases, 117, 146, 201, 370, 432.
Carnegie Institution of Washington, acknowledgment, 375, 432.
Centroblepharoplast, in *Chilomastix*, 123, 124, 137; in *Leidyopsis*, 104, 111; in *Menoidium* and *Trichomonas*, 123, 124, 459; in *Trichomitus*, 27, 31, 37; in *Trichonympha*, 47, 48, 53, 60, 62, 70, 71; connection of with neuromotor fibers in flagellates, 345.
Centrosome, in *Chilomastix*, 118, 122, 124; in *Endamoeba coli*, 323; in *Giardia*, 206; in *Pentatrichomonas*, 379; in *Streblomastix*, 7, 16; in *Trichomitus*, 23 31, 32; in *Trichonympha*, 47 60, 71.
Cercaria tenax, 157, 158.
Cercomonas, 147–150.
crassicauda, 147, 148.
davainei, 152, *passim*.
hominis, 147, 148, *passim*.
intestinalis, 160, 163.
longicauda, 148, 149, 150.
obliqua, 152.
parva, 147, 148, *passim*.
Chemotropism, 496.

Chilomastix, 148, 151, 205, 210, 216, 406, 496. *See also* under Cysts, Cytoplasm, Mitosis, etc.
davainei, 147, 152, 155, 208, 366, 376.
mesnili, 117–144, 147, 151.
China Medical Board, acknowledgment, 370.
Chromatoidal bodies, 130, 176, 178, 316, 506, 508, 517.
Chromomeres, 67.
Chromophile ridge, 179, 187, 507, 508, 513.
Chromosomes, in *Councilmania decumani*, 441; in *C. lafleuri*, 177, 306, 435; in *C. muris*, 439, 510; in *Chilomastix*, 134; in *Endamoeba coli*, 177, 319, 320; in *Euglena viridis*, 466–467; in *Leidyopsis*, 108, 109, 111; in man, 305; in *Menoidium*, 455, 466, 467; in *Streblomastix*, 11; in *Trichomitus*, 32, 33, 34; in *Trichonympha*, 67, 72–77, 81; continuity of, 76; differentiation of species by, 529.
Cilia, in *Balantidium*, 265, 266, 270, 273, 284, 289; in *Paramaecium*, movement of isolated pieces of, 350; in *Streblomastix*, 14; in *Trichonympha*, 48, 50; in *Trichonympha* and *Paramaecium*, 345; adoral, 284, 287, 291, 293; adoral ciliary rootlets, 286.
Cockroach, host for *Endamoeba*, 432.
Comparison of the Cysts of Endamoeba coli and Councilmania lafleuri in Congo Red, 365–371.
Congo red, reactions to, of cysts, 365–371.
Cooper, H. N., acknowledgment, 334.
Copromonas major, 458, 463.
subtilis, 458.
Cort, W. W., acknowledgment, 245.
Councilmania, generic diagnosis, 187, 431–445, 493; key to species of, 443; comparison of species, 523, 530.
decumani, 411, 433, 438, 439–443, 491, 495, *passim*.
lafleuri, 169–198; activities, 171; free stage, 173, 186; encysted, 175, 186; dimensions, 180; budding and escape of amoebulae, 181; occurrence, 183; culture and inoculation, 185; cysts, viability of, 185; distinctions, 186; comparison of cysts in, with *Endamoeba coli*, 365–371; infection of rodents with, 409–430, 497, 502, 506, *passim*.
muris, 411, 438–439, 491, 495, *passim*.
Cross-infection, experiments in, with parasitic amoeba, 517.
Craigia, 148, 149, 150, 431.
hominis, 150.
Craspedomonadina, direction of torsion in, 9.
Critical Review of the Nomenclature of Human Intestinal Flagellates, Cercomonas, Chilomastix, Trichomonas, Tetratrichomonas, and Giardia, 145–168.
Culture of *Councilmania*, 185; of *Leishmania*, 447; of *Paramaecium*, 336.
Cummings, W. J., acknowledgment, 334.
Cutaneous reaction, 477–488; table, 480.
Cysts, in *Chilomastix*, 129, 130; in *Councilmania decumani*, 443, 513; in *C. lafleuri*, 179, 185; in *C. muris*, 439, 503–507; in *Endamoeba coli*, 179, 313–332; in *Endamoeba coli*, and *Councilmania lafleuri*, 365–371, 432, 435, 437; in *Endamoeba dysenteriae*-infected rats, 414; in *E. ratti*, 516, 517; in *Giardia enterica*, 217, 218, 219.
degenerative changes in, 314.
feeding experiments with, 413–421.
difference in viability of, 422, 425.
Cytolysis, 182.
Cytopharynx, in *Paramaecium*, 342.
Cytoplasm, in *Balantidium*, 263; in *Chilomastix*, 121; in *Councilmania lafleuri*, 176, 181, 182; in *C. muris*, 505; in *Giardia*, 204; in *Pentatrichomonas*, 381; in *Streblomastix*, 7; in *Trichomitus*, 24.
Cytopyge, 280.
Cytostome, in *Balantidium*, 260, 267, 276; in *Chilomastix*, 126; in *Giardia*, 204; in *Paramaecium*, 342, 344; in *Pentatrichomonas*, 380; in *Trichomitus*, 26.
Devescovina, 78, 373.
Dexiotropism in polymastigotes, 9.
Dientamoeba fragilis, 170.
Diet, milk only, antagonistic to normal amoebic infection, 521, 522; meat only, suitable, 521, 522.
Dimorphus muris, 160, 163.
Dinenympha, 9, 78.
gracilis, 2, 14, 216.
Dinoflagellata, direction of torsion in, 9.
Diplodinium, 21, 60, 267.
ecaudatum, 273, 279, 280, *passim*, 335.
Diplozoic organization, in flagellates, 29.
Distinguishing Characteristics of the Parasitic Amoeba of Culture Rats and Mice, 489–544.
Domonoske, A. B., acknowledgment, 334.
Drew, A. D., acknowledgment, 2.

Ectoplasm, in *Balantidium*, 263, 266, 267; in *Councilmania lafleuri*, 173; in *C. muris* and *C. decumani*, 499; in *Endamoeba ratti*, 515; in *Leidyopsis*, 102, 107, 111; in *Trichonympha*, 48, 49, 52, 61, 62.
Ely, C. L., acknowledgment, 302.
Embadomonas, 151.
  intestinalis, 147.
Encystment, 402, 527–528; in *Councilmania decumani*, 512; in *C. lafleuri*, 175, 186; in *C. muris*, 503; in *Endamoeba ratti*, 516; in *Giardia*, 217, 219, 221, 222; in *Trichomitus termitidis*, 36.
Endamoeba dysenteriae in the Lymph Glands of Man in Hodgkin's Disease, 309–312.
Endamoeba blattae, 524.
  coli, 170, 173, 177, *passim*, 324, 365–371, 411–430, 441, 490, 491, 507, *passim*.
  dysenteriae, 118, 129, 130, 156, 170, 173, *passim*, 301–307, 309–312, 366, 410–430, 504–505, *passim*; conclusions from feeding experiments, 413–421; chronic type in rats, 414.
  gingivalis, 306.
  histolytica, 322, 497.
  legeri, 322, 323.
  muris decumani, 491.
  nana, 366.
  ratti, 411, 491, 515–530.
  williamsi, 184.
Endolimax bnetschlii, 129, 130.
  nana, 170, 411, 416, 418, 422.
Endoplasm, in *Balantidium*, 282; in *Councilmania lafleuri*, 174; in *C. muris* and *C. decumani*, 499; in *Endamoeba ratti*, 515; in *Leidyopsis*, 105; in *Menoidium*, 450; in *Trichonympha*, 48, 56, 60.
Endosome, in euglenoids, 463–464.
Entamoeba muris, 170, 434.
  tetragena, 434.
  undulans, 384.
Environment, not determining factor in parasitic amoebae, 519, 523.
Epsom salt, purgative in rodent experiments, 403, 405, 492, 517.
Euglena agilis, 451, 458.
  viridis, 452, 462, 465, 466.
Enplotes, 64, 79.
  patella, 270, 278, 281.
  worcesteri, 283.
Eutrichomastix, 9, 33.
Experimental Infection of Rats and Mice with the Common Intestinal Amoeba of Man, 409–430.
Extra-nuclear motor apparatus, definition of, 21.
Extracts, alcoholic and alkaline, of *Leishmania*, 479; table, 480.
Faeces, routine examinations of, in man and rodents, 401, 402, 408.
Fibers, in *Balantidium:* diverging, 268, circumoesophageal, 285, 289, 290, radial, 286, 287, 289; in *Chilomastix*, peristomal, 125; in *Giardia*, 211, 212; in *Leidyopsis*, oblique, 103, 111, as in *Trichonympha*, 52, 53; in *Paramaecium*, peripheral, 341; of cytosome and cytopharynx in *Paramaecium*, 342–343, 352; fibrillar system conductile, 347–351.
Fission, binary, in *Endamoeba coli*, 317; in *Leidyopsis*, 107; in *Menoidium*, 457, 460; in *Pentatrichomonas*, 382; in *Streblomastix*, 10, 11; in *Trichomitus*, 35; in *Trichonympha*, 44, 61; usual in motile amoebae, 527.
  multiple, in *Pentatrichomonas*, 382; in *Streblomastix*, 12, 13; in *Trichomitus*, 35, 37; usual in parasitic amoeba, 527; plane of, in *Giardia enterica*, 215.
Flagella, in *Chilomastix*, 128; in *Devescovina*, 373; in *Giardia*, 206, 207, 208, 418, 457; in *Heteronema acus*, 457; in Hypermastigina, 373; in *Leidyopsis*, 103, 110, 111; in *Pentatrichomonas*, 378, 383; in *Streblomastix*, 7, 8, 14, 16; in *Trichomitus*, 27, 37; in *Trichomonas*, 158, 378; in *Trichonympha*, 47, 48, 50, 110; in *Tritrichomonas*, 378; bases of classification in flagellates, 146, 158.
Flagellates, susceptibility to biochemical changes, 29, 43; diplozoic organization, 29; of human intestine, 117–144, 145–168, 199–234; method of feeding, 391–400.
Food. *See* Nutrition.
Force, J. N., acknowledgment, 477.
Fowler, Margaret B., acknowledgment, 171, 375.
Giardia, comparison with *Streblomastix*, 7; with *Trichomitus*, 22, 29; with *Trichonympha*, 75; with *Chilomastix*, 118, 119, 136, 137; description of genus, 160–164; specific differences, 222, 225.
  agilis, 161, 200.
  alata, 161, 162.
  cuniculi, 222.
  duodenalis, 162, 200.
  enterica, 162, 163, 164, 199–234, 366, 418.
  intestinalis, 147, 211.
  lamblia, 164.
  microti, 162, 200, 206, 222.
  muris, 162, 163, 200, 204, *passim*, 406.
  sanguinis, 200.

Glycogen, vacuole, 178; in *Councilmania lafleuri*, 435; in *C. muris*, 439, 503, 505; in cytoplasm of *Endamoeba coli* at encystment, 317; in *E. ratti*, 517.
Gradients, axial, in *Paramaecium*, 347–348, 352.
Gyrodinium corallinum, 58.
Hall, R. P., 447–476.
Heterochromosome, in *Trichonympha*, use of term, 72.
Heteronema acus, 457.
Hexamastix ardin delteili, 377.
Hexamita duodenalis, 160, 163.
Hexamitidae, 136, 137, 216, 217.
Hexamitus, 30.
  muris, 406.
Hodgkin's disease, 303, 304, 305, 309–312.
Holomastigotes, 9, 15, 70.
Holomastigotoides, 78.
Hydrogen-ion concentration, in caeca of rats, 520.
Hypermastigina, 80, 81, 112, 373.
Immunization to *Leishmania*, 478, 483.
Intestine, human, parasites of, *see* Amoeba; Flagellates.
Intradesmose, in *Councilmania lafleuri*, 177; in *C. muris*, 509; found in *Endamoeba dysenteriae*, *E. coli*, and *Councilmania lafleuri*, 304, 319; characteristic in amoebae, 529.
Iodamoeba buetschlii, 174, 175, 366, 411, 416, 418, 422, 526.
Isogamy, in flagellates, 461.
Isotricha prostoma, 272, 273.
Joenia, 78.
Karyosome, in *Councilmania decumani*, 441, 512; in *C. lafleuri*, 174, 176, 432, 433, 435, 437; in *C. muris*, 439, 502, 506; in *Endamoeba coli*, 317; in *E. ratti*, 517; "nucleolo-centrosome," 464.
Kessel, J. F., 401–408, 409–430, 489–544.
Kofoid, C. A., 145–168; acknowledgments, 245, 315, 334, 401, 411, 448, 477, 491.
Kofoid, C. A., Boyers, L. M., and Swezy, Olive, 301–312.
Kofoid, C. A., and Swezy, Olive, 1–20, 21–40, 41–98, 99–116, 117–144, 169–198, 199–234, 373–390.
Kofoid, C. A., Swezy, Olive, and Kessel, J. F., 431–445.
Kornhauser, S. I., acknowledgment, 119.
Lamblia, 161, 162, *passim*.
  intestinalis, 200.
Legge, R. T., acknowledgment, 171.
Leidyopsis, morphology, 100–101; relationships, 110–111; generic diagnosis, 111; pseudopodial method of feeding, 391–400.
  sphaerica, 99–116; surface ridges, 103, 111.
Leiotropism, in polymastigotes, 9, 15.
Leishmania tropica, 477–488.
  infantum, 477–488.
Leishmaniosin, preparation of, 478.
Literature cited, on: *Balantidium*, 295–297; *Councilmania*, 188, 371, 445; experimental infection with rats, 408, 426–427; *Leishmania*, 485–486; *Menoidium*, 469–472; mitosis, 138, 225–227, 312, 325–326; nomenclature of human intestinal flagellates, 165–168; *Paramaecium*, 242, 353–355; parasites of the termites, 17, 37, 82–83, 113, 400; pathological agent in Hodgkin's disease, 312; *Pentatrichomonas*, 388–389; parasitic amoebae of rats and mice, 531–533.
Locomotion, in *Councilmania decumani*, 512; in *C. lafleuri*, 171–173, 435; in *C. muris*, 495–501, 512; in *Endamoeba ratti*, 515–516; in *Pentatrichomonas*, 382–385; in *Trichonympha*, 43, 391–400.
Lophomonas, 78.
Lung infection, among rats, 414, 425.
Lymph glands, presence of parasite in, 309–312.
McDonald, J. D., 243–300.
Macronucleus in *Balantidium coli* and *B. suis*, 259–260, 263, 282.
Macrostoma mesnili, 151.
McVey, C. L., acknowledgment, 171.
Magnesium sulphate, purgative in experiments with rodents, 408.
Mastigophora, direction of torsion in, 9.
Medical zoology, value to, of cross-infection, 423.
Megastoma entericum, 161, 163, 200, 211.
Menoidium incurvum, 447–476.
Methods of Obtaining Amoeba-free Rats for Experimental Infection with Intestinal Amoeba, 401–408.
Mice, infection experiments with, 406, 407, 414, 489–544; facultative carriers of amoebiasis, 423.
Micro-dissection experiments, 235; on *Euplotes patella*, 292; on *Paramaecium*, 349.
Micro-Injection of Paramaecium, 235–242, 336, 346.

Micronucleus, in *Balantidium*, 263, 283.
Microtus californicus, 222.
Mitosis in Endamoeba dysenteriae in the Bone Marrow in Arthritis Deformans, 301–307.
Mitosis, similar in all species of parasitic amoeba, 522; in *Chilomastix*, 133, 137; in *Councilmania decumani*, 441, 514; in *C. lafleuri*, 177; in *C. muris*, 439, 509; in *Endamoeba coli*, 317–325, comparison with *Councilmania lafleuri*, 322; in *E. dysenteriae*, 301–307; in *Euglena* and *Menoidium*, 467, 468; in *Giardia*, 199–234; in human cell, 304–305; in *Leidyopsis*, 108, 111; in *Noctiluca*, 71; in *Pentatrichomonas*, 382; in the Rhizopoda and Metazoa, 301; in *Streblomastix*, 10, 14; in *Trichomitus*, 27, 31, 34; in *Trichomonads*, 27; in *Trichonympha*, 44, 47, 61, 64, 65, 69, 72, figures illustrating, 73.
Mitosis and Fission in the Active and Encysted Phases of Giardia enterica (Grassi) of Man, with a Discussion of the Method of Origin of Bilateral Symmetry in the Polymastigote Flagellates, 199–234.
Morphology and Binary Fission of Menoidium incurvum (Fres.) Klebs, 447–476.
Morphology, in *Balantidium*, 262, 263; in *Chilomastix*, 119; differences in, between *Endamoeba coli* and *Councilmania lafleuri*, 315; in *Giardia*, 202, 223; in *Leidyopsis*, 100; in *Menoidium*, 450; in *Paramaecium*, 338; in *Pentatrichomonas*, 378; in *Streblomastix*, 4; in *Trichomitus*, 23; in *Trichonympha*, 44, 81; a criterion in identification, 306, 310; constancy of, in transference of amoebae, 517, 519.
Motorium, in *Balantidium*, 285, 289.
Multiple fission. *See* Fission, multiple.
Mus decumanus, 491.
  musculus, 492.
  norvegicus albinus, 492.
  rattus, 410, 434, 491.
Myonemes, in *Streblomastix*, 7, 13, 14, 15, 16; in *Trichonympha*, 55, 56, 57; in *Leidyopsis*, 105, 111.
Naegleria gruberi, 323.
National Council of Research, acknowledgment, 119.
Neuromotor apparatus, definition and extent of, in flagellates, 21, 22; function of, 47–49, 350–351; in *Balantidium*, 284–287; in *Chilomastix*, 123, 133, 137; in *Diplodinium*, 288; in *Euplotes*, 288, 289, 335; in *Giardia*, 203, 205; in *Leidyopsis*, 102, 111; in *Menoidium*, 451, 468; in *Paramaecium*, 333–364; in *Streblomastix*, 7; in *Trichomitus*, 26, 27, isolated, 30, during mitosis, 31, 32; in *Trichonympha*, 47–48, 62, 81.
Neuromotor Apparatus of Paramaecium, 333–364.
Noctiluca, mitosis in, 71.
Nomenclature, human intestinal flagellates, 145–168.
Novy, F. E., acknowledgment, 477.
Nuclear membrane, in *Councilmania decumani*, 441, 512; in *C. lafleuri*, 437, 443; in *C. muris*, 439, 502, 506; in *Endamoeba ratti*, 516; in *Leidyopsis*, 109, 111.
Nucleolo-centrosome, theory of, 464.
Nucleus, in *Chilomastix*, 121, 122, 131, 137; in *Copromonas*, 462; in *Councilmania decumani*, 433, 441, 513; in *C. lafleuri*, 174, 175, 435; in *C. muris*, 439, 502, 509; in *Endamoeba coli*, 317, 320; in *Leidyopsis*, 106, 107; in *Menoidium*, 453, 462; in *Pentatrichomonas*, 381; in *Streblomastix*, 10, 16; in *Trichomitus*, 24, 29, 37; in *Trichonympha*, 57, 58, 81.
Nutrition, in *Balantidium*, 282; in *Councilmania muris*, 496; in *Leidyopsis*, 101, 391–400; in *Menoidium*, 452; in *Paramaecium*, 237, 238; in *Streblomastix*, 7, 13; in *Trichomitus*, and *Trichonympha*, 23, 56, 81, 391, *passim;* in trichonymphid flagellates, 391–400; comparisou between milk only and meat only diets in parasitic amoebae, 521–522.
On Balantidium coli (Malmsten) and Balantidium suis (Sp. Nov.), with an Account of their Neuromotor Apparatus, 243–300.
On the Free, Encysted, and Budding Stages of Councilmania lafleuri, a Parasitic Amoeba of the Human Intestine, 169–198.
On the Genus Councilmania, Budding Intestinal Amoeba Parasitic in Man and Rodents, 431–445.
On the Morphology and Behavior of Pentatrichomonas ardin delteili Derrieu and Raynaud), 373–390.
On the Morphology and Mitosis of Chilomastix mesnili (Wenyon), a Common Flagellate of the Human Intestine, 117–144.
Operculum, 45.
Ophyroscolex, 267.

Organelles, locomotor, in *Balantidium*, 290; in *Diplodinium*, 290; in *Euplotes*, 290; in *Leidyopsis*, 103; in *Trichonympha*, 50; doubling of, in promitosis, 374.
Osmosis, in *Streblomastix*, 13.
Oxyrrhis marina, 464.
Parabasal body, in *Chilomastix*, 126; in *Giardia*, 200, 209, 210; in *Trichomitus*, 28, 29, 31, 32, 37.
Paradesmose, in *Chilomastix*, 118, 124, 133, 137; in *Copromonas*, 458; in *Euglena agilis*, 458, 459; in *Giardia*, 215, 224, 459; in *Leidyopsis*, 107, 108, 111; in *Menoidium*, 458, 459; in *Trichomitus*, 29, 32, 33, 37, 71; in *Trichomonas*, 71, 459; in *Trichonympha*, 47, 62, 71, 81; in contradistinction to intradesmose, 177, 304, 319.
Paramaecium, 235–242, 249, 281; neuromotor apparatus of, 333–364.
Parasites of the termites, studies on, 1–116; of human intestine, *see* Amoeba; Flagellates; specificity of, or transference, 421–425, 519, 526; morphological constancy of, in cross-infection, 424, 425, 519; varied occurrence, 431; possible source of infection of, in man, 526.
Parastyle, 127.
Pellicle, in *Balantidium*, 263, 265; in *Paramaecium*, 349.
Pentatoma, 72.
Pentatrichomonas, found only in man, 432.
  ardiu delteili, 373–390.
  bengalensis, 377.
Peristome, 275, 277.
Peromyscus maniculatus, 222.
Personympha flagellata, 216.
Pigs, hosts for *Balantidium*, 246, 293.
Plasmolysis, 265.
Plastids, in endoplasm of *Menoidium*, 450.
Polymastigina, 15, 16, 81, 373.
Polymastigote flagellates, evolution of, 373.
Protozoa, 1, 42. *See also*, Amoeba; Flagellates.
Pseudopodia, in *Councilmania decumani*, 439, 496, 497–501, 525; in *C. lafleuri*, 172, 173, 432, 435, 497; in *C. muris*, 496, 497–501, 525; in *Endamoeba coli*, 497; in *E. dysenteriae*, 410; in *E. histolytica*, 497; in *E. ratti*, 515; in *Trichonympha*, 391–400; as means of nutrition, 391–400; as determining factor in establishing species, 522–524, 525.
Pseudopodial Method of Feeding by Trichonymphid Flagellates Parasitic in Wood-eating Termites, 391–400.
Pseudosynapsis (pseudotelosynapsis) in *Leidyopsis*, 111; in *Trichonympha*, interpretation of, 72–77, 81.
Pseudotrichonympha, 78.
Purgatives, in experiments with rats: castor oil, 403; epsom salt, 403, 406, 408.
Pyrsonema, 78.
Pyrsonympha, 9, 78, 373.
  verteus, 2, 14.
Rabbits, in experiments with *Leishmania*, 477–488.
Rats, infection experiments with, 401–430, 489–544; carriers of amoebiasis, 423.
Rattus norvegicus, 411, 491, 492.
Rees, C. W., 235–242, 333–364.
Rhabdomonas incurva, 448.
Rhizoplast, in *Chilomastix*, 118, 131, 132, 137; in *Giardia*, 206; in *Menoidium*, 451, 452; in *Pentatrichomonas*, 379, 387; in *Streblomastix*, 7, 16; in *Trichomitus*, 31, 32, 37; in *Trichonympha*, 52.
Rhodes, R. C., acknowledgment, 448.
Sarah Berliner Research Fellowship, acknowledgment, 315.
Schizonts, arrangement of, in *Trichomonas*, 11.
Sexual behavior, absence of, in *Streblomastix*, 10, in *Trichomitus*, 35, and in *Trichonympha*, 72.
Skin Reaction to Extracts of Leishmania tropica and Leishmania infantum, 477–488.
Smith, Inez, acknowledgment, 411, 432.
Specificity of parasites, 421.
Sphaerita, as parasite in amoeba, 496.
Spiral groove, in *Chilomastix*, 118, 121, 135, 137.
Spireme in *Chilomastix*, 134.
Spirostomum, 334, 349.
Spirotrichonympha, 9, 14.
Staining, *intra vitam*, in *Paramaecium*, 347. *See also* Congo red, reactions of cysts to; Technique.
Stentor coeruleus, 269, 334.
Streblomastigidae, 15, 16.
Streblomastix, morphology, 4–10; relationships, 14–15; generic diagnosis, 15.
  strix, 1–20; occurrence with *Trichonympha*, 43.
Striations, longitudinal, on *Menoidium incurvum*, 450.

Studies on the Parasites of the Termites:
I. On Streblomastix strix, a Polymastigote Flagellate with a Linear Plasmodial Phase, 1–20.
II. On Trichomitus termitidis, a Polymastigote Flagellate with a Highly Developed Neuromotor System, 21–40.
III. On Trichonympha campanula, Sp. Nov., 41–98.
IV. On Leidyopsis sphaerica, Gen. Nov., Sp. Nov., 99–116.
Swezy, Olive, 313–332, 391–400; acknowledgment, 411, 477, 491.
Taylor, C. V., acknowledgment, 334.
Technique, 3–4, 171, 245, 336–338, 401–408, 411–412, 492.
Termites, parasites of, studies on, 1–116.
Termopsis angusticollis, 2, 15, 16, 22, 36, 37, 43, 100, 111, 392; amount of infection in single individual, 2, 22.
Tetratrichomonas, 9; synonym for *Trichomonas*, 159.
buccalis, 156, 158, 159.
hominis, 156.
parisi, 159.
prowazeki, 159.
Torsion, direction and structural expression of, in polymastigotes, 9.
Tradescantia virginica, 465.
Tricercomonas intestinalis, 147.
"Trichomastix" condition, defined, 158.
Trichomitopsis, 36, 37.
Trichomitus, 9; short-lived in cultures, 23; nutrition in, 23; biochemical changes of environment, 29; paradesmose in, 71.
parvus, 36, 37.
termitidis, 21–40.
Trichomonas, 9, 21, 23, 151, 155, *passim*; arrangement of schizonts in, 11; multiple fission in, 13, 14; paradesmose in, 71; centroblepharoplast in, 123, 124; distinct from *Pentatrichomonas*, 377; in rats, 405, 406.
augusta, 24, 160.
buccalis, 156, 159.
confusa, 156.
hominis, 155, 158, *passim*, 374, 375, 376, 377–386.
intestinalis, 147, 156, 158.
muris, 406.
pulmonalis, 156, 158.
sensu latu, 160; *sensu strictu*, 159.
vaginalis, 156, 158, *passim*.
Trichonympha, 2, 10, 22, 23; agility, 44, 393; key to, 80; relationship with *Leidyopsis*, 110, 391–400.
agilis, first of genus discovered, 44; ridges on, 49; flagella of, 50; anterior part of, 60, 61; *minore* and *maggiore*, 69, 78.
campanula, 41–98, 100; similarity with *Paramaccium*, 339, with *Menoidium*, 467.
hertwigi, 46, 47, 61, 70, 72, 78.
Trichonymphidae, 80, 81, 112.
Tritrichomonas, 160, 378.
augusta, 160.
Trypanosoma, 162.
Tyzzer, E. E., acknowledgment, 477.
Vacuoles, food, in *Trichomitus*, 24; contractile, in *Balantidium*, 263, 280, 281.
Virgulina pirenula, 157.
Wagener, Edna H., 477–488.
Walker, E. L., acknowledgment, 411, 491.

ERRATA

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